RC
268.5
U55
no.162
1979

ional Cancer Institute

CARCINOGENESIS

Technical Report Series
NO. 162

1979

BIOASSAY OF
2, 4-DIAMINOTOLUENE
FOR POSSIBLE CARCINOGENICITY

CAS No. 95-80-7

NCI-CG-TR-162

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
National Institutes of Health

Library
National Institutes of Health
Bethesda, Maryland 20014

BIOASSAY OF
2,4-DIAMINOTOLUENE
FOR POSSIBLE CARCINOGENICITY

Carcinogenesis technical report series

United States

Carcinogenesis Testing Program
Division of Cancer Cause and Prevention
National Cancer Institute
National Institutes of Health
Bethesda, Maryland 20014

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
National Institutes of Health

DHEW Publication No. (NIH) 79-1718

BIOASSAY OF
2,4-DIAMINOTOLUENE
FOR POSSIBLE CARCINOGENICITY

Carcinogenesis Testing Program
Division of Cancer Cause and Prevention
National Cancer Institute
National Institutes of Health

FOREWORD: This report presents the results of the bioassay of 2,4-diaminotoluene conducted for the Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute (NCI), National Institutes of Health, Bethesda, Maryland. This is one of a series of experiments designed to determine whether selected chemicals have the capacity to produce cancer in animals. A negative result, in which the test animals do not have a greater incidence of cancer than control animals, does not necessarily mean that a test chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of circumstances. A positive result demonstrates that a test chemical is carcinogenic for animals under the conditions of the test and indicates that exposure to the chemical is a potential risk to man. The actual determination of the risk to man from chemicals found to be carcinogenic in animals requires a wider analysis.

CONTRIBUTORS: This bioassay of 2,4-diaminotoluene was conducted at the NCI Frederick Cancer Research Center (FCRC) (1), Frederick, Maryland, operated for NCI (2) by Litton Bionetics, Inc.

The manager of the bioassay at FCRC was Dr. B. Ulland, the toxicologist was Dr. E. Gordon, and Drs. R. Cardy and D. Cresia compiled the data. Ms. S. Toms was responsible for management of data, Mr. D. Cameron for management of histopathology, Mr. L. Callahan for management of the computer branch, and Mr. R. Cypher for management of the facilities. Mr. A. Butler performed the computer services. Necropsies were performed by Drs. B. Ulland, R. Schueler, R. Ball, and R. Cardy. Histopathologic evaluations for rats were performed by Dr. Cardy, and histopathologic evaluations for mice were performed by Dr. M. D. Reuber. The diagnoses included in this report represent the interpretations of Drs. Cardy and Reuber.

Animal pathology tables and survival tables were compiled at EG&G

Mason Research Institute (3). Statistical analyses were performed by Dr. J. R. Joiner (4) and Ms. P. L. Yong (4), using methods selected for the bioassay program by Dr. J. J. Gart (5).

The chemicals used in this bioassay were analyzed at FCRC by Dr. W. Zielinsky. The chemical narrative and analyses were reviewed and approved by Dr. W. Lijinsky (1).

This report was prepared at Tracor Jitco (4) under the direction of NCI. Those responsible for the report at Tracor Jitco were Dr. C. R. Angel, Acting Director of the Bioassay Program; Dr. S. S. Olin, Deputy Director for Science; Dr. J. F. Robens, toxicologist; Dr. R. L. Schueler, pathologist; Dr. G. L. Miller, Ms. L. A. Owen, Ms. M. S. King, and Mr. W. D. Reichardt, bioscience writers; and Dr. E. W. Gunberg, technical editor, assisted by Ms. Y. E. Presley.

The following scientists at NCI were responsible for evaluating the bioassay experiment, interpreting the results, and reporting the findings: Dr. Kenneth C. Chu, Dr. Cipriano Cueto, Jr., Dr. J. Fielding Douglas, Dr. Richard A. Griesemer, Dr. Thomas E. Hamm, Dr. William V. Hartwell, Dr. Morton H. Levitt, Dr. Harry A. Milman, Dr. Thomas W. Orme, Dr. A. R. Patel, Dr. Sherman F. Stinson, Dr. Jerrold M. Ward, and Dr. Carrie E. Whitmire.

---

(1) Frederick Cancer Research Center, P.O. Box B, Frederick, Maryland.

(2) Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute, National Institutes of Health, Bethesda, Maryland.

(3) EG&G Mason Research Institute, 1530 East Jefferson Street, Rockville, Maryland.

(4) Tracor Jitco, Inc., 1776 East Jefferson Street, Rockville, Maryland.

(5) Mathematical Statistics and Applied Mathematics Section, Biometry Branch, Field Studies and Statistics, Division of Cancer Cause and Prevention, National Cancer Institute, National Institutes of Health, Bethesda, Maryland.

SUMMARY

A bioassay of 2,4-diaminotoluene for possible carcinogenicity was conducted by administering the test chemical in feed to F344 rats and B6C3F1 mice.

Groups of 50 rats of each sex were administered 2,4-diaminotoluene at one of two doses, initially either 125 or 250 ppm, for 40 weeks. Because of excessive depression in the amount of mean body weight gained in both the low- and high-dose groups, doses were then reduced to 50 and 100 ppm, respectively. Administration of 50 ppm to the low-dose groups was continued for 63 weeks, and surviving animals in these groups were then killed. Surviving animals in the high-dose males and females administered 100 ppm were killed at the end of 39 and 44 weeks, respectively, due to morbidity. The time-weighted average dose was 79 ppm for the low-dose males and females for 103 weeks, 176 ppm for the high-dose males for 79 weeks, and 171 ppm for the high-dose females for 84 weeks. Matched controls consisted of 20 untreated rats of each sex.

Groups of 50 mice of each sex were administered 2,4-diaminotoluene at one of two doses, either 100 or 200 ppm, for 101 weeks. Matched controls consisted of 20 untreated mice of each sex. Surviving mice were killed at the end of administration of the test chemical.

Mean body weights of dosed male and female rats and mice were lower than those of corresponding controls and were dose related except for the low-dose male mice, for which mean body weights were only slightly lower than those of controls. Mortality was not dose related in either the male or female mice, but was dose related in both the male and female rats. Survival was decreased and lesions of hepatonephrotoxicity were observed in the animals administered the 2,4 diaminotoluene.

In the rats, hepatocellular carcinomas or neoplastic nodules occurred at incidences that were dose related in both the males (P = 0.014) and the females (P = 0.008). In direct comparisons of incidences of the tumors in control and dosed groups, the incidence in the high-dose male group had a P value of 0.026 (males: controls 0/20, low-dose 5/49, high-dose 10/50; females: controls 0/20, low-dose 0/50, high-dose 6/49). The significance of the occurrence of these tumors in both the male and female rats was supported by high incidences of associated nonneoplastic lesions of the liver in the dosed goups and by low incidences of

liver tumors in historical-control male or female F344 rats at the same laboratory.

In addition, carcinomas or adenomas of the mammary gland occurred in the female rats at incidences that were dose related (P = 0.001) and in direct comparisons were higher in the dosed groups (P less than 0.001) than in the control group (controls 1/20, low-dose 38/50, high-dose 41/50).

In the male rats, fibromas of the subcutaneous tissue occurred at incidences that were dose related (P = 0.004) and in direct comparisons were higher in the dosed groups (P less than or equal to 0.020) than in the control group (controls 0/20, low-dose 15/30, high-dose 19/50).

In the mice, hepatocellular carcinomas occurred in the females at incidences that were dose related (P = 0.002) and in direct comparisons were higher in the dosed groups (P less than or equal to 0.007) than in the control group (controls 0/19, low-dose 13/47, high-dose 18/46). In addition, lymphomas occurred at a significant incidence (P less than 0.001) in the low-dose female mice (controls 2/19, low-dose 29/47, high-dose 11/46). No tumors occurred at significantly increased incidences in the dosed male mice.

Under the conditions of this bioassay, 2,4-diaminotoluene was carcinogenic for F344 rats, inducing hepatocellular carcinomas or neoplastic nodules in both males and females and carcinomas or adenomas of the mammary gland in females. The test chemical was also carcinogenic for female B6C3F1 mice, inducing hepatocellular carcinomas. The incidence of lymphomas in the female mice suggested that these tumors also may have been related to administration of the test chemical.

TABLE OF CONTENTS

Page

I. Introduction 1

II. Materials and Methods 3

- A. Chemical 3
- B. Dietary Preparation 3
- C. Animals 4
- D. Animal Maintenance 5
- E. Subchronic Studies 7
- F. Chronic Studies 9
- G. Clinical and Pathologic Examinations 10
- H. Data Recording and Statistical Analyses 13

III. Results - Rats 19

- A. Body Weights and Clinical Signs (Rats) 19
- B. Survival (Rats) 19
- C. Pathology (Rats) 22
- D. Statistical Analyses of Results (Rats) 26

IV. Results - Mice 29

- A. Body Weights and Clinical Signs (Mice) 29
- B. Survival (Mice) 29
- C. Pathology (Mice) 32
- D. Statistical Analyses of Results (Mice) 35

V. Discussion 37

VI. Bibliography 41

APPENDIXES

Appendix A Summary of the Incidence of Neoplasms in Rats Administered 2,4-Diaminotoluene in the Diet 43

Table A1 Summary of the Incidence of Neoplasms in Male Rats Administered 2,4-Diaminotoluene in the Diet 45

Page

Table A2 Summary of the Incidence of Neoplasms in Female Rats Administered 2,4-Diaminotoluene in the Diet 49

Appendix B Summary of the Incidence of Neoplasms in Mice Administered 2,4-Diaminotoluene in the Diet 53

Table B1 Summary of the Incidence of Neoplasms in Male Mice Administered 2,4-Diaminotoluene in the Diet 55

Table B2 Summary of the Incidence of Neoplasms in Female Mice Administered 2,4-Diaminotoluene in the Diet 59

Appendix C Summary of the Incidence of Nonneoplastic Lesions in Rats Administered 2,4-Diaminotoluene in the Diet 63

Table C1 Summary of the Incidence of Nonneoplastic Lesions in Male Rats Administered 2,4-Diaminotoluene in the Diet 65

Table C2 Summary of the Incidence of Nonneoplastic Lesions in Female Rats Administered 2,4-Diaminotoluene in the Diet 70

Appendix D Summary of the Incidence of Nonneoplastic Lesions in Mice Administered 2,4-Diaminotoluene in the Diet 75

Table D1 Summary of the Incidence of Nonneoplastic Lesions in Male Mice Administered 2,4-Diaminotoluene in the Diet 77

Table D2 Summary of the Incidence of Nonneoplastic Lesions in Female Mice Administered 2,4-Diaminotoluene in the Diet 80

Appendix E Analyses of the Incidence of Primary Tumors in Rats Administered 2,4-Diaminotoluene in the Diet 83

Page

Table E1 Analyses of the Incidence of Primary Tumors in Male Rats Administered 2,4-Diaminotoluene in the Diet 85

Table E2 Analyses of the Incidence of Primary Tumors in Female Rats Administered 2,4-Diaminotoluene in the Diet 98

Appendix F Analyses of the Incidence of Primary Tumors in Mice Administered 2,4-Diaminotoluene in the Diet 109

Table F1 Analyses of the Incidence of Primary Tumors in Male Mice Administered 2,4-Diaminotoluene in the Diet 111

Table F2 Analyses of the Incidence of Primary Tumors in Female Mice Administered 2,4-Diaminotoluene in the Diet 115

TABLES

Table 1 2,4-Diaminotoluene Subchronic Feeding Studies in Rats and Mice 8

Table 2 2,4-Diaminotoluene Chronic Feeding Studies in Rats 11

Table 3 2,4-Diaminotoluene Chronic Feeding Studies in Mice 12

FIGURES

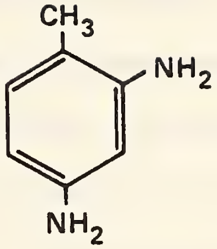
Figure 1 Growth Curves for Rats Administered 2,4-Diaminotoluene in the Diet 20

Figure 2 Survival Curves for Rats Administered 2,4-Diaminotoluene in the Diet 21

Figure 3 Growth Curves for Mice Administered 2,4-Diaminotoluene in the Diet 30

Figure 4 Survival Curves for Mice Administered 2,4-Diaminotoluene in the Diet 31

I. INTRODUCTION



2,4-Diaminotoluene

2,4-Diaminotoluene (CAS 95-80-7; NCI C02305) is a widely used industrial intermediate. Most of this chemical produced in the United States is converted to toluene diisocyanate for use in the synthesis of polyurethanes (Backus, 1974). 2,4-Diaminotoluene is also an intermediate for the synthesis of dyes used for textiles, fur, leather, biological stains and indicators, spirit varnishes and wood stains, and pigments (International Agency for Research on Cancer, 1978; Society of Dyers and Colourists, 1971). In addition, it has been used as a component of oxidation-type hair dye formulations (Wall, 1972). Two hundred and thirty-three million pounds of 2,4-diaminotoluene were produced in the United States in 1976 (United States International Trade Commission, 1977a). In addition, 356,000 pounds were imported in that year (United States International Trade Commission, 1977b).

The carcinogenicity of 2,4-diaminotoluene was first reported by Ito et al. (1969), using a small number of rats administered the chemical in the diet. Similar studies, sponsored by the National Cancer Institute (Weisburger et al., in press), suggested that 2,4-diaminotoluene was carcinogenic in both rats and mice. For these reasons the chemical was selected for additional study in the Carcinogenesis Testing Program using expanded protocols.

II. MATERIALS AND METHODS

A. Chemical

2,4-Diaminotoluene was obtained from Eastman Organic Chemicals, Eastman Kodak Co., Rochester, New York. This material is a light-brown solid. The melting point was 97°C, which was consistent with the value of 99°C given in the literature (Weast, 1974-1975). Mass spectral analysis gave a base peak for its molecular ion at m/e 122 and a peak of equivalent abundance at m/e 121. Elemental analysis showed 68.9% carbon, 8.4% hydrogen, and 23.4% nitrogen (theoretical: 68.9% C, 8.2% H, and 23.0% N). Purity was determined by gas-liquid chromatography to be greater than 99.9%, with up to six minor contaminants. The infrared spectrum was consistent with the chemical structure of the compound.

The test material was stored at 5°C until used.

B. Dietary Preparation

Test diets containing 2,4-diaminotoluene were prepared at

Frederick Cancer Research Center (FCRC) every 1 to 1½ weeks in 6- to 12-kg batches at appropriate doses. A known weight of the chemical was first mixed with an equal weight of autoclaved Wayne® Sterilizable Lab Meal contained 4% fat (Allied Mills, Inc., Chicago, Ill.), using a mortar and pestle. The mixing was continued with second and third additions of feed, and final mixing was performed with the remaining quantity of feed for a minimum of 15 minutes in a Patterson-Kelly® twin-shell blender. The diets were routinely stored at 5°C until used.

C. Animals

Male and female F344 (Fischer) rats and B6C3F1 mice were obtained as 4-week-old weanlings, all within 3 days of the same age, from the NCI Frederick Cancer Research Center (Frederick, Md.). The animals were housed within the test facility for 2 weeks and were then assigned four rats to a cage and five mice to a cage on a weight basis for each cage of animals of a given species and sex. Male rats used in the chronic study weighed 90 to 105 g, averaging at least 100 g; the female rats, 80 to 95 g, averaging at least 90 g; the male mice, 18 to 22 g, averaging at least 19.5 g; and the female mice, 17 to 21 g, averaging at least 18.5 g. Individual animals were identified by ear punch.

D. Animal Maintenance

The animals were housed in polycarbonate cages (Lab Products, Inc., Garfield, N.J.), 19 x 10½ x 8 inches for the rats and 11½ x 7½ x 5 inches for the mice, which were suspended from aluminum racks (Scientific Cages, Inc., Bryan, Tex.) and were covered by nonwoven polyester-fiber 12-mil-thick filter paper (Hoeltge, Inc., Cincinnati, Ohio). The bedding used was Absorb-dri® hardwood chips (Northeastern Products, Inc., Warrenburg, N.Y.). The feed supplied was presterilized Wayne® Sterilizable Lab Meal containing 4% fat, provided ad libitum in suspended stainless steel hoppers and replenished at least three times per week. Water, acidified to pH 2.5, was supplied ad libitum from glass bottles with sipper tubes (Lab Products, Inc.) suspended through the tops of the cages.

The contaminated bedding was disposed of through an enclosed vacuum line that led to a holding tank from which the bedding was fed periodically into an incinerator. The cages were sanitized twice per week and the feed hoppers twice per month at 82 to 88°C in a tunnel-type cagewasher (Industrial Washing Corp., Mataway, N. J.), using the detergents, Clout® (Pharmacal Research Laboratories, Greenwich, Conn.) or Oxford D'Chlor (Oxford Chemicals, Atlanta, Ga.). The bottles and sipper tubes were sanitized at 82 to 88°C in a tunnel-type bottle washer (Consolidated Equipment Supply Co., Mercersburg, Pa.) three times per week, using a Calgen Commercial Division detergent (St. Louis, Mo.). The racks for the cages were sanitized at or above 82°C in a rack washer (Consolidated Equipment Supply Co.) once per month, using the Calgen Commercial Division detergent, and the filter paper was changed at the same time.

The animal rooms were maintained at 22 to 24°C and 45 to 55% relative humidity. Incoming air was passed through a filter of 65% efficiency and a bag filter of 95% efficiency at the intake and expelled without recirculation through a "Z"-type roughing filter of 30% efficiency and a bag system of 90 to 95% efficiency at the exhaust (American Air Filters, Louisville, Ky.; Mine Safety Appliances, Pittsburgh, Pa.). Room air was changed 15 times per hour. The air pressure was maintained negative to a clean hallway and positive to a return hallway. Fluorescent lighting was provided automatically on a 12-hour-per-day cycle.

Rats administered 2,4-diaminotoluene and their corresponding controls were housed in the same room as rats on feeding studies of the following chemicals:

(CAS 85-44-9) phthalic anhydride
(CAS 95-53-4) o-toluidine hydrochloride

Mice administered 2,4-diaminotoluene and their corresponding controls were housed in the same room as mice on feeding studies of the following chemicals:

(CAS 156-62-7)	calcium cyanamide
(CAS 999-81-5)	chlorocholine chloride
(CAS 19010-66-3)	lead dimethyldithiocarbamate
(CAS 86-30-6)	N-nitrosodiphenylamine
(CAS 88-96-0)	phthalamide
(CAS 120-62-7)	piperonyl sulfoxide
(CAS 137-17-7)	2,4,5-trimethylaniline

E. Subchronic Studies

Subchronic feeding studies were conducted to estimate the maximum tolerated doses (MTD's) of 2,4-diaminotoluene, on the basis of which two concentrations (referred to in this report as "low" and "high" doses) were selected for administration in the chronic studies. Groups of five rats and five mice of each sex were fed diets containing 2,4-diaminotoluene at one of several doses for 7 weeks, followed by 1 week of observation, and groups of five control animals of each species and sex were administered basal diet only. Each animal was weighed twice per week. Table 1 shows the doses fed, the survival of animals in each dosed group at the end of the study and the mean body weights of dosed animals at week 7, expressed as percentages of mean body weights of controls. At the end of the 8-week period, all animals were killed using CO_2 and necropsied.

Table 1. 2,4-Diaminotoluene Subchronic Feeding Studies in Rats and Mice

	Male		Female	
Dose (ppm)	Surviv- al (a)	Mean Weight at Week 7 as % of Control	Surviv- al (a)	Mean Weight at Week 7 as % of Control
RATS				
0	5/5	100	5/5	100
250	5/5	96	5/5	91
500	5/5	82	5/5	93
1,000	5/5	59	5/5	80
2,000	0/5		1/5	61
3,000	0/5		0/5	
MICE				
0	5/5	100	5/5	100
100	5/5	103	5/5	103
200	5/5	101	5/5	93
300	5/5	86	5/5	87
500	5/5	80	5/5	85
700	5/5	84	5/5	79
1,000	3/5	74	5/5	76

(a) Number surviving/number in group.

In rats receiving 1,000 ppm, slight increases in hematopoiesis and cytoplasmic vacuolation of hepatocytes were seen in both sexes. Small amounts of bile duct hyperplasia occurred in males at this dose. No clinical or histopathologic findings were reported for the male mice at 700 ppm or for the females at 1,000 ppm.

Ten percent depression in body weight was a major criterion for the estimation of MTD's. The doses required to produce this response were determined by the following procedure: first, least square regressions of mean body weights versus days on study were used to estimate mean body weights of each of the dosed groups at day 49. Next, probits of the percent weights of the dosed groups at day 49 relative to weights of corresponding control groups were plotted against the logarithms of the doses, and least squares regressions fitted to the data were used to estimate the doses required to induce 10% depression in weight. The low and high doses for the chronic studies were set at 125 and 250 ppm for rats; and 100 and 200 ppm for mice.

F. Chronic Studies

The test groups, doses administered, and duration of the chronic feeding studies are shown in tables 2 and 3. Due to excessive depression in the amount of mean body weight gained in the dosed male and female rats, doses for the low- and high-dose groups were reduced to 50 and 100 ppm, respectively, after week 40.

G. Clinical and Pathological Examinations

All animals were observed twice daily. Observations for sick, tumor-bearing, and moribund animals were recorded daily. Clinical examination and palpation for masses were performed each month, and the animals were weighed at least once per month. Moribund animals and animals that survived to the end of the bioassay were killed using CO_2 and necropsied.

The pathologic evaluation consisted of gross and microscopic examination of major tissues, major organs, and all gross lesions. The tissues were preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The following tissues were examined microscopically: skin, lungs and bronchi, trachea, bone marrow (femur), spleen, lymph nodes (mesenteric and submandibular), thymus, heart, salivary glands (parotid, sublingual, and submaxillary), liver, pancreas, esophagus, stomach (glandular and

Table 2. 2,4-Diaminotoluene Chronic Feeding Studies in Rats

Sex and Test Group	Initial No. of Animals (a)	2,4-Diaminotoluene in Diet (b) (ppm)	Time on Study (weeks)	Time-Weighted Average Dose (e) (ppm)
Male				
Matched-Control	20	0	103	
Low-Dose	50	125	40	
		50	63	79
High-Dose	50	250	40	
		100	39(c)	176
Female				
Matched-Control	20	0	103	
Low-Dose	50	125	40	
		50	63	79
High-Dose	50	250	40	
		100	44(d)	171

(a) All animals were 6 weeks of age when placed on study.

(b) Test and control diets were provided *ad libitum* 7 days per week.

(c) Administration of test diet for the high-dose males was terminated at the time indicated and all the animals were killed, due to morbidity.

(d) Administation of test diet for the high-dose females was terminated at the time indicated and all animals except four were killed, due to morbidity.

(e) Time-weighted average dose = $\frac{\Sigma(\text{dose in ppm x no. of weeks at that dose})}{\Sigma(\text{no. of weeks receiving each dose})}$

Table 3. 2,4-Diaminotoluene Chronic Feeding Studies in Mice

Sex and Test Group	Initial No. of Animals (a)	2,4-Diaminotoluene in Diet (b) (ppm)	Time on Study (weeks)
Male			
Matched-Control	20	0	101
Low-Dose	50	100	101
High-Dose	50	200	101
Female			
Matched-Control	20	0	101
Low-Dose	50	100	101
High-Dose	50	200	101

(a) All animals were 6 weeks of age when placed on study.

(b) Test and control diets were provided ad libitum 7 days per week.

nonglandular), small and large intestine, kidney, urinary bladder, pituitary, adrenal, thyroid, parathyroid, testis, prostate, mammary gland, uterus, ovary, brain (cerebrum and cerebellum), and all tissue masses. Peripheral blood smears also were made for all animals, whenever possible.

Necropsies were also performed on all animals found dead, unless precluded in whole or in part by autolysis or cannibalization. Thus, the number of animals from which particular organs or tissues were examined microscopically varies and does not necessarily represent the number of animals that were placed on study in each group.

H. Data Recording and Statistical Analyses

Pertinent data on this experiment have been recorded in an automatic data processing system, the Carcinogenesis Bioassay Data System (Linhart et al., 1974). The data elements include descriptive information on the chemicals, animals, experimental design, clinical observations, survival, body weight, and individual pathologic results, as recommended by the International Union Against Cancer (Berenblum, 1969). Data tables were generated for verification of data transcription and for statistical review. These data were analyzed using the appropriate statistical techniques described in this section. Those analyses of the experimental results that bear on the possibility of carcinogenicity are discussed in the statistical narrative section.

Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier (1958) and are presented in this report in the form of graphs. Animals were statistically censored as of the time that they died of other than natural causes or were found to be missing; animals dying from natural causes were not statistically censored. Statistical analyses for a possible dose-related effect on survival used the method of Cox (1972) for testing two groups for equality and Tarone's (1975) extensions of Cox's methods for testing for a dose-related trend. One-tailed P values have been reported for all tests except the departure from linearity test, which is only reported when its two-tailed P value is less than 0.05.

The incidence of neoplastic or nonneoplastic lesions has been given the ratio as of the number of animals bearing such lesions at a specific anatomic site (numerator) to the number of animals in which that site is examined (denominator). In most instances, the denominators included only those animals for which that site was examined histologically. However, when macroscopic examination was required to detect lesions prior to histologic sampling (e.g., skin or mammary tumors), or when lesions could have appeared at multiple sites (e.g., lymphomas), the denominators consist of the numbers of animals necropsied.

The purpose of the statistical analyses of tumor incidence is to determine whether animals receiving the test chemical developed a significantly higher proportion of tumors than did the control animals. As a part of these analyses, the one-tailed Fisher exact test (Cox, 1970) was used to compare the tumor incidence of a control group with that of a group of dosed animals at each dose level. When results for a number of dosed groups (k) are compared simultaneously with those for a control group, a correction to ensure an overall significance level of 0.05 may be made. The Bonferroni inequality (Miller, 1966) requires that the P value for any comparison be less than or equal to 0.05/k. In cases where this correction was used, it is discussed in the narrative section. It is not, however, presented in the tables, where the Fisher exact P values are shown.

The Cochran-Armitage test for linear trend in proportions, with continuity correction (Armitage, 1971), was also used. Under the assumption of a linear trend, this test determines if the slope of the dose-response curve is different from zero at the one-tailed 0.05 level of significance. Unless otherwise noted, the direction of the significant trend is a positive dose relationship. This method also provides a two-tailed test of departure from linear trend.

A time-adjusted analysis was applied when numerous early deaths resulted from causes that were not associated with the formation of tumors. In this analysis, deaths that occurred before the first tumor was observed were excluded by basing the statistical tests on animals that survived at least 52 weeks, unless a tumor was found at the anatomic site of interest before week 52. When such an early tumor was found, comparisons were based exclusively on animals that survived at least as long as the animal in which the first tumor was found. Once this reduced set of data was obtained, the standard procedures for analyses of the incidence of tumors (Fisher exact tests, Cochran-Armitage tests, etc.) were followed.

When appropriate, life-table methods were used to analyze the incidence of tumors. Curves of the proportions surviving without an observed tumor were computed as in Saffiotti et al. (1972). The week during which an animal died naturally or was sacrificed was entered as the time point of tumor observation. Cox's methods of comparing these curves were used for two groups; Tarone's extension to testing for linear trend was used for three groups. The statistical tests for the incidence of tumors which used life-table methods were one-tailed and, unless otherwise noted, in the direction of a positive dose relationship. Significant departures from linearity (P less than 0.05, two-tailed test) were also noted.

The approximate 95 percent confidence interval for the relative risk of each dosed group compared with its control was calculated from the exact interval on the odds ratio (Gart, 1971). The relative risk is defined as p_t/p_c where p_t is the true binomial probability of the incidence of a specific type of tumor in a dosed group of animals and p_c is the true probability of the spontaneous incidence of the same type of tumor in a control group. The hypothesis of equality between the true proportion of a specific tumor in a dosed group and the proportion in a control group corresponds to a relative risk of unity. Values in excess of unity represent the condition of a larger proportion in the dosed group than in the control.

The lower and upper limits of the confidence interval of the relative risk have been included in the tables of statistical analyses. The interpretation of the limits is that in approximately 95% of a large number of identical experiments, the true ratio of the risk in a dosed group of animals to that in a control group would be within the interval calculated from the experiment. When the lower limit of the confidence interval is greater than one, it can be inferred that a statistically significant result (P less than 0.025 one-tailed test when the control incidence is not zero, P less than 0.050 when the control incidence is zero) has occurred. When the lower limit is less than unity, but the upper limit is greater than unity, the lower limit indicates the absence of a significant result while the upper limit indicates that there is a theoretical possibility of the induction of tumors by the test chemical, which could not be detected under the conditions of this test.

III. RESULTS - RATS

A. Body Weights and Clinical Signs (Rats)

Starting at about week 6, mean body weights of dosed male and female rats were markedly lower than those of corresponding controls, and were dose related (figure 1). The incidences of tissue masses and of wasting were higher in the dosed groups than in the control groups; other clinical signs, such as corneal opacity, were common to both groups.

B. Survival (Rats)

Estimates of the probabilities of survival for male and female rats administered 2,4-diaminotoluene in the diet at the doses of this bioassay, together with those for the matched controls, are shown by the Kaplan and Meier curves in figure 2. In each sex, the result of the Tarone test for positive dose-related trend in mortality is significant (P less than 0.001). A departure from linear trend is indicated (P less than 0.001 in males and P = 0.009 in females) due to the relatively steep decrease in survival in the dosed groups as compared with the controls.

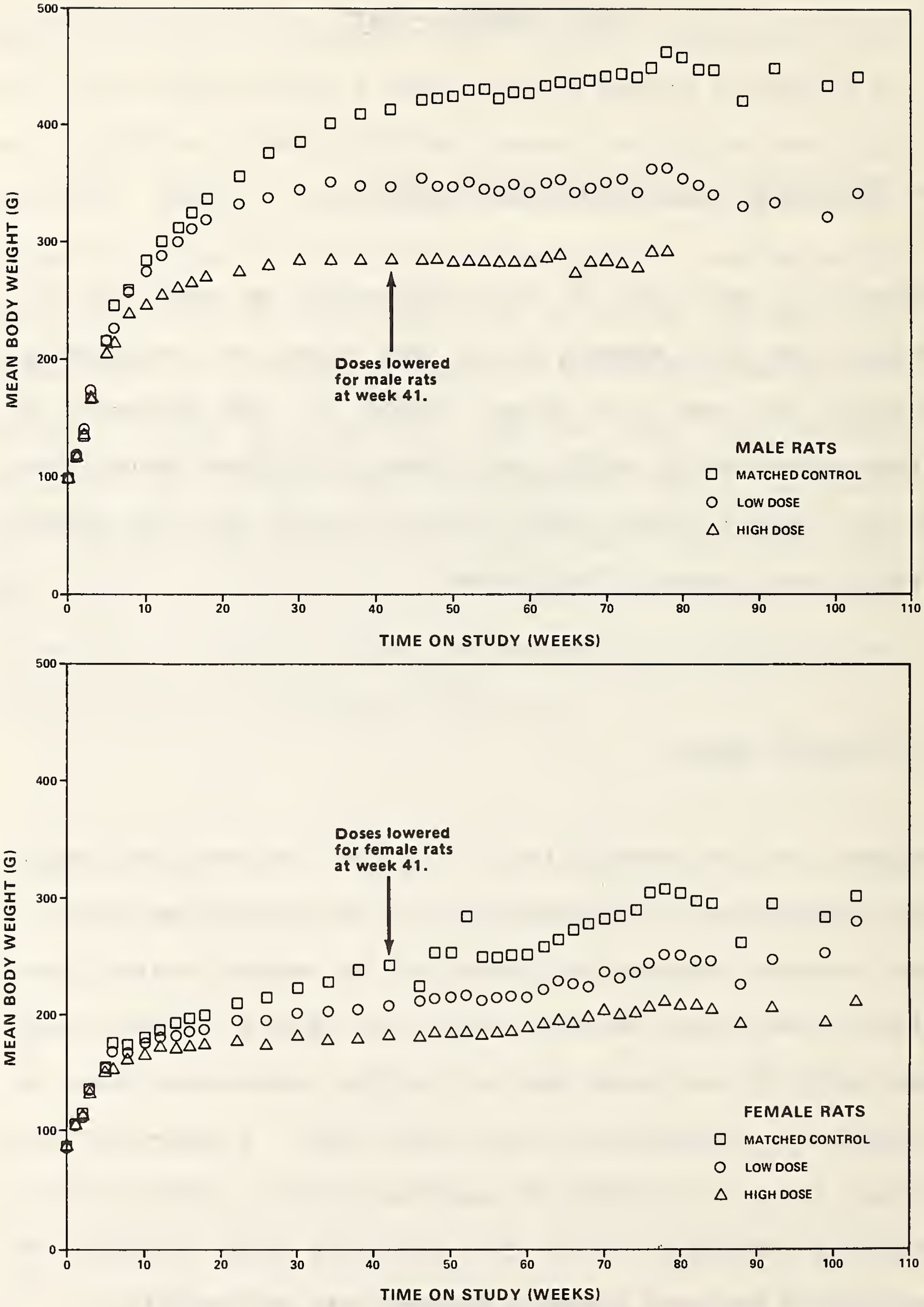


Figure 1. Growth Curves for Rats Administered 2,4-Diaminotoluene in the Diet

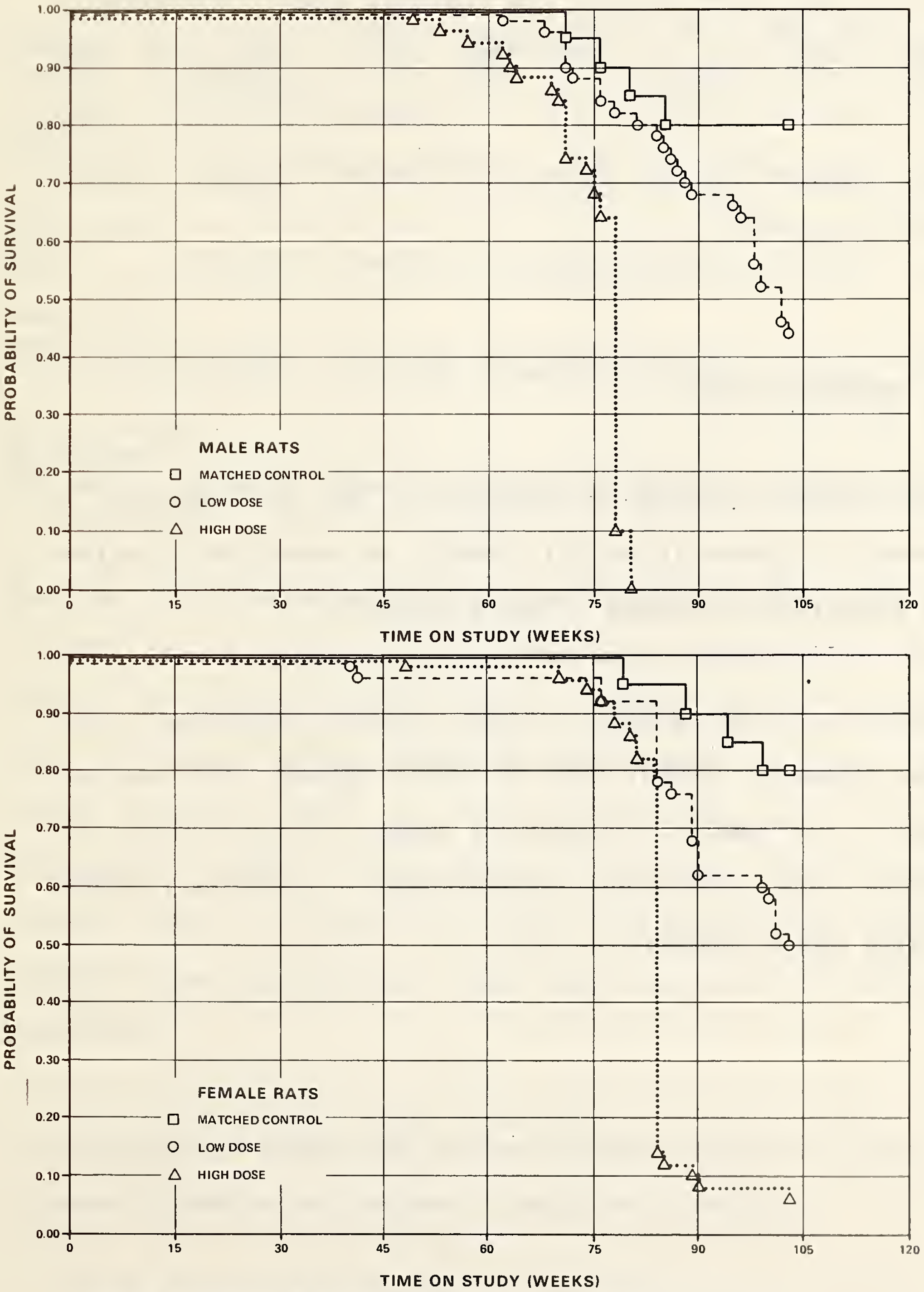


Figure 2. Survival Curves for Rats Administered 2, 4-Diaminotoluene in the Diet

In male rats, 32/50 (64%) of the high-dose group, 42/50 (84%) of the low-dose group, and 18/20 (90%) of the control group were still alive at week 78 on study. In females, 46/50 (92%) of each dosed group, and all 20 of the control group were still alive at week 78 on study.

C. Pathology (Rats)

Histopathologic findings on neoplasms in rats are summarized in Appendix A, tables A1 and A2; findings on nonneoplastic lesions are summarized in Appendix C, tables C1 and C2.

In male rats, the incidence of liver neoplasia was higher in the dosed groups of animals than in control groups. The same was true in the females, although the numbers of tumors were much smaller. The incidences of the proliferative and primary hepatic lesions were as follows:

Hepatic Lesion	Males Control	Males Low Dose	Males High Dose	Females Control	Females Low Dose	Females High Dose
Hepatocellular Carcinoma	0/20(0%)	3/49(6%)	6/50(12%)	0/20(0%)	0/50(0%)	3/49(6%)
Neoplastic Nodule	0/20(0%)	2/49(4%)	5/50(10%)	0/20(0%)	0/50(0%)	3/49(6%)
Number of Animals Bearing Tumors	0/20(0%)	5/49(10%)	10/50(20%)	0/20(0%)	0/50(0%)	6/49(12%)
Foci or Areas of Cellular Alteration	2/20(10%)	25/49(51%)	36/50(72%)	1/20(5%)	23/50(46%)	42/49(86%)

There was an increased incidence of proliferative lesions generally believed to be associated with the hepatocarcinogenesis that occurred in response to the chemical. These lesions consisted mainly of foci of cellular alteration described as clear-cell type (Squire and Levitt, 1975), but a few basophilic foci were scattered throughout. In many of these lesions, there was a good deal of nuclear atypia. In nearly all cases in which frank hepatic neoplasias were encountered, there were also foci of cellular alteration.

The incidence of benign and malignant mammary tumors was greatly increased in females and increased in males as shown below:

Mammary Tumors	Males Control	Males Low Dose	Males High Dose	Females Control	Females Low Dose	Females High Dose
Carcinoma	0/20(0%)	1/50(2%)	0/50(0%)	0/20(0%)	9/50(18%)	8/50(16%)
Other Malignant Tumors	0/20(0%)	2/50(4%)	0/50(0%)	0/20(0%)	1/50(2%)	1/50(2%)
Fibroadenoma	0/20(0%)	3/50(6%)	4/50(8%)	1/20(5%)	26/50(52%)	29/50(58%)
All Other Adenomas	0/20(0%)	1/50(2%)	1/50(2%)	0/20(0%)	13/50(26%)	18/50(36%)
Number of Animals Bearing Tumors	0/20(0%)	5/50(10%)	5/50(10%)	1/20(5%)	38/50(76%)	42/50(84%)

The most common mammary tumor by far was the fibroadenoma, which as a group could not be interpreted as life threatening. None of the mammary tumors metastasized, although there might have been metastases in dosed animals had their survival been better.

Other types of tumors, though appearing less frequently than liver and mammary tumors, nevertheless may have been related to exposure to the chemical. Tumors which fell into this category included lung tumors, squamous-cell carcinomas of the skin and the preputial gland, pancreatic acinar-cell adenomas, and subcutaneous fibromas and fibrosarcomas in both sexes, and mesotheliomas in males.

A number of nonneoplastic lesions were encountered in most animals examined. They occurred without apparent relation to compound administration. The only endemic disease seen with any frequency was mild chronic respiratory disease. It was not of sufficient severity to affect longevity.

Chronic renal disease normally seen in aging F344 rats was found to be much more severe and of earlier onset in dosed animals than in control animals. The effect was most marked in males and is considered to be an important result of chronic toxicity which may have contributed to the decreased survival of the dosed animals.

Corresponding to the renal disease was a high incidence of associated secondary hyperparathyroidism in low- and high-dose males; and, in addition to the cases tabulated, there were numerous others that were suggestive of the same condition.

In the liver, dosed animals exhibited a wide range of chemically induced morphologic alterations which ranged from scattered foci of lipidosis and cellular alteration to severe, diffuse toxic degenerative changes.

Based on the histopathologic examination, 2,4-diaminotoluene at the doses used was hepatonephrotoxic. The compound was carcinogenic for F344 rats under the conditions of this study, inducing proliferative hepatic lesions and neoplasms in both sexes. It induced a high incidence of benign and malignant tumors of the mammary gland in females and an incidence above that of the controls in males. In addition, tumors of the subcutis appeared to be associated with administration of the compound.

D. Statistical Analyses of Results (Rats)

Tables E1 and E2 in Appendix E contain the statistical analyses of the incidences of those primary tumors that occurred in at least two animals of one group and at an incidence of at least 5% in one or more than one group.

In male rats, the result of the Cochran-Armitage test for positive dose-related trend in the incidence of fibromas of the subcutaneous tissue is significant (P = 0.009). The Fisher exact test shows that the incidence in either the low- or high-dose group is significantly higher than that in the control group (P = 0.020 and P = 0.004, respectively). The statistical conclusion is that the incidence of this tumor in the male rats is associated with the administration of 2,4-diaminotoluene. In females, the result of the Cochran-Armitage test is significant (P = 0.009). However, the Fisher exact comparison of the incidences in the high-dose and control groups indicates a P value of 0.026, which is above the 0.025 level required for significance when the Bonferroni inequality criterion is used for multiple comparison.

The results of the Cochran-Armitage test are significant for the incidence of lipomas of the subcutaneous tissue (P = 0.017) and for the incidence of mesothelioma of all sites (P = 0.042) in male rats, but the results of the Fisher exact test are not significant.

The result of the Cochran-Armitage test for positive dose-related trend in the incidence of male rats with either hepatocellular carcinoma or neoplastic nodules is significant (P = 0.014), but the Fisher exact comparison of the incidences in the high-dose and control groups indicates a P value of 0.026, which is above the 0.025 level required for significance when the Bonferroni inequality criterion is used for multiple comparison. In females, the result of the Cochran-Armitage test on the incidence of these liver tumors is significant (P = 0.008), but the results of the Fisher exact test are not significant. The incidences of combined neoplastic nodules and hepatocellular carcinomas in historical-control male and female F344 rats at this laboratory are 7/285 (2.5%) and 0/285 (0%), respectively, compared with 10/50 (20%) and 6/49 (12%) in the male and female high-dose groups in this study. Early deaths may have reduced the incidences of the tumors in the high-dose groups.

In females, the results of the Cochran-Armitage test for the incidence of adenoma of the mammary gland and the combined incidence of adenoma and carcinoma of the mammary gland are significant (P less than 0.001). Departures from linear trend are observed (P less than or equal to 0.002), due to the relatively steep increase in the incidence of tumors in the dosed groups. The results of the Fisher exact test show that the incidences in each dosed group are significantly higher than those in the control group (P less than 0.001). The statistical conclusion is that these incidences of mammary gland tumors in female rats are associated with the administration of 2,4-diaminotoluene.

Significant results in the negative direction are observed in the incidence of carcinomas of the pituitary in male rats and in the incidence of hematopoietic tumors in female rats. That the incidence is higher in the control group than in the dosed groups may be due to the earlier mortality of the dosed rats.

In summary of the statistical findings, the incidences of fibromas of the subcutaneous tissue in male rats and of tumors of the mammary gland in females are associated with the administration of 2,4-diaminotoluene.

IV. RESULTS - MICE

A. Body Weights and Clinical Signs (Mice)

Mean body weights of high-dose male and both low- and high-dose female mice were lower than those of corresponding controls, and those of the females were dose related (figure 3). Other clinical signs, such as tissue masses and wasting, occurred at low incidences, and were common to dosed and control groups.

B. Survival (Mice)

Estimates of the probabilities of survival for male and female mice administered 2,4-diaminotoluene in the diet at the doses of this bioassay, together with those for the matched controls, are shown by the Kaplan and Meier curves in figure 4. The result of the Tarone test for dose-related trend in mortality is not significant in either sex.

In male mice, 43/50 (86%) of the high-dose group, 45/50 (90%) of the low-dose group, and 18/20 (90%) of the control group lived to the end of the study. In females, 39/50 (78%) of the high-dose,

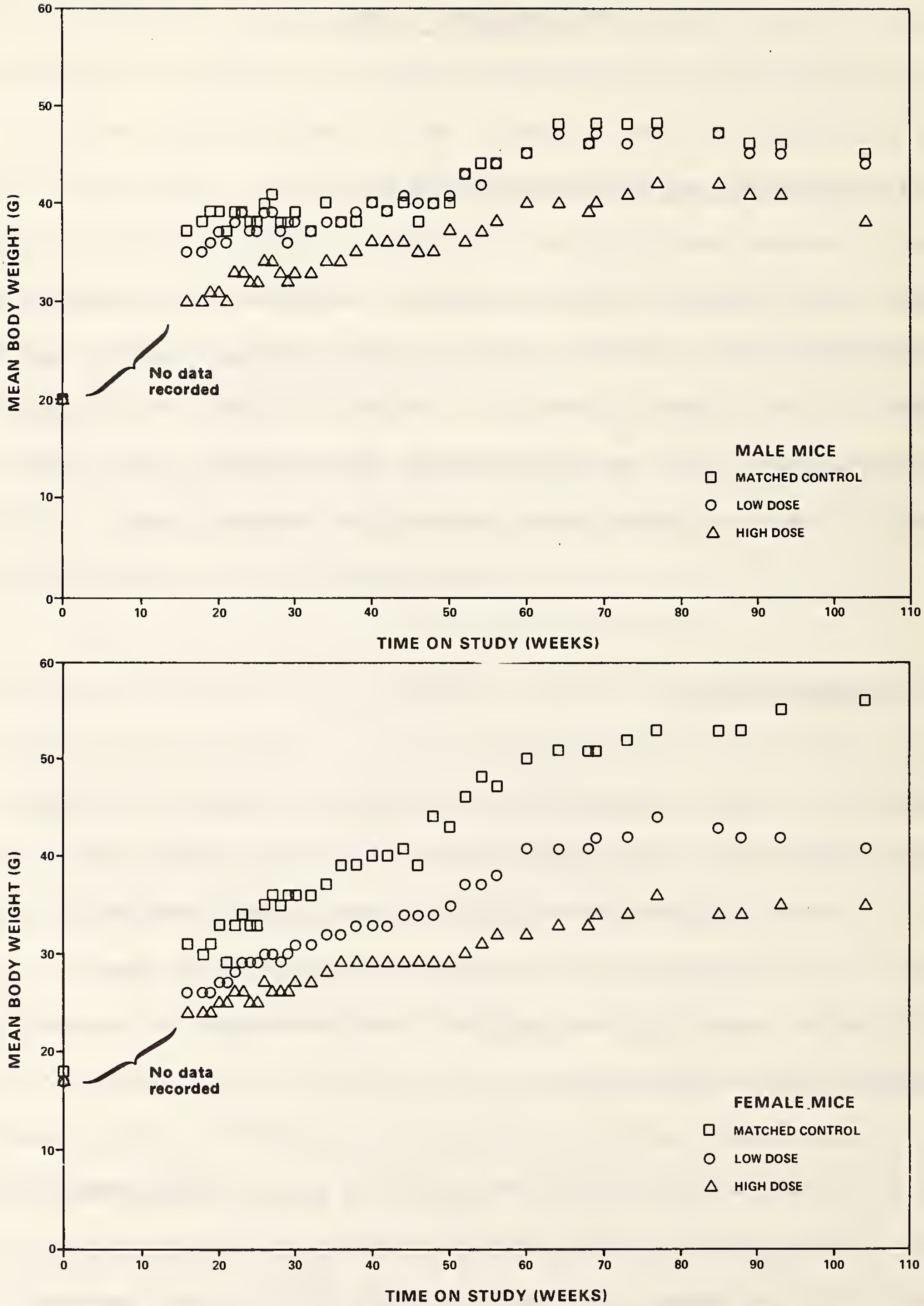


Figure 3. Growth Curves for Mice Administered 2, 4-Diaminotoluene in the Diet

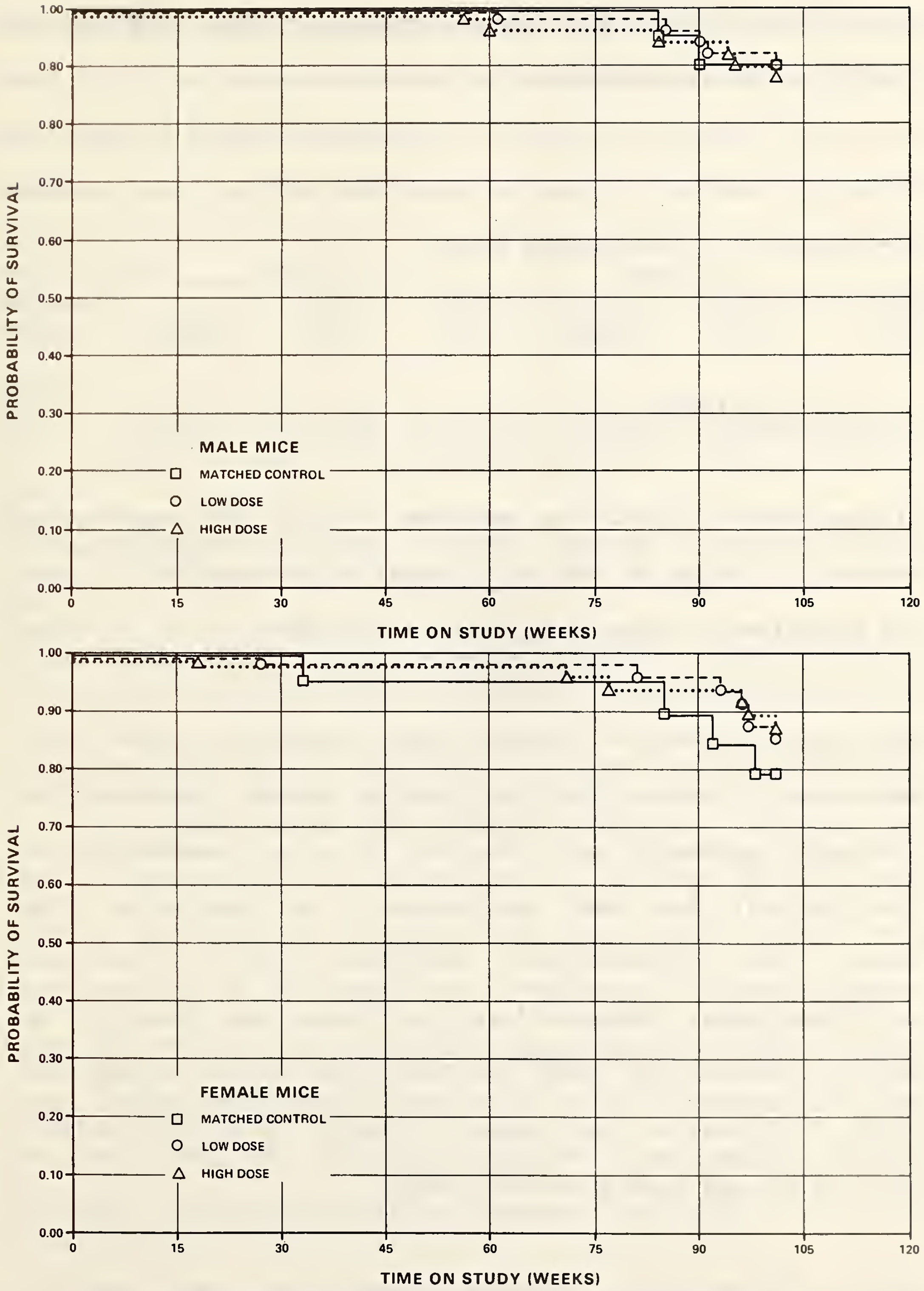


Figure 4. Survival Curves for Mice Administered 2, 4-Diaminotoluene in the Diet

group, 40/50 (80%) of the low-dose group, and 15/20 (75%) of the control group lived to the end of the study.

Sufficient numbers of mice of each sex were at risk for the development of late-appearing tumors.

C. Pathology (Mice)

Histopathologic findings on neoplasms in mice are summarized in Appendix B, tables B1 and B2; findings on nonneoplastic lesions are summarized in Appendix D, tables D1 and D2.

Neoplasms in mice were observed more frequently in the liver, hematopoietic system, lung, and vascular system. Hepatocellular carcinomas generally were observed on gross examination and histologically had the characteristics of anaplasia. They usually were differentiated carcinomas, but a few were undifferentiated. Hepatocellular carcinomas were found in the males: controls 5/20 (25%), low-dose 17/50 (34%), and high-dose 13/49 (27%), and in the females: controls 0/19 (0%), low-dose 13/47 (28%), and high-dose 18/46 (39%).

Hepatic nonneoplastic lesions associated with chemical administration were diffuse, and nodular hyperplasia occurred in dosed males and females but not in corresponding controls. The incidences of these nonneoplastic lesions of the liver were as follows:

Hepatic Non-neoplastic Lesions	Males			Females		
	Control	Low Dose	High Dose	Control	Low Dose	High Dose
Hyperplasia, NOS	0/20(0%)	11/50(22%)	26/49(53%)	0/20(0%)	8/47(17%)	5/46(11%)
Hyperplasia, Diffuse	0/20(0%)	8/50(16%)	4/49(8%)	0/20(0%)	13/47(28%)	0/46(0%)
Hyperplastic Nodule	0/20(0%)	6/50(12%)	4/49(8%)	0/20(0%)	9/47(19%)	22/46(48%)

Most of the hematopoietic neoplasms were lymphomas, except for those in low-dose female mice, which included leukemias. Lymphomas were either lymphosarcomas or reticulum-cell sarcomas histologically; leukemias were lymphocytic. Mice with these tumors tended not to have tumors of the liver and lung. Neoplasms of the hematopoietic system in the mice (not including hemangiomas and hemangiosarcomas of the lymph node) were found in the males (controls 2/20 (4%); low- dose 15/50 (30%); high-dose 8/49 (16%)) and females (controls 2/19 (10%); low-dose 29/47 (62%); high-dose 1/46 (24%)).

Neoplasms of the lung and vascular system were slightly increased in male mice given 2,4-diaminotoluene. Carcinomas of the lung were well-differentiated papillary adenocarcinomas or poorly differentiated carcinomas. In the male mice, 0/20 (0%) control, 9/50 (18%) low-dose, and 41/49 (84%) high-dose animals had carcinomas of the lung. Hemangiomas and hemangiosarcomas in male mice receiving 2,4-diaminotoluene most often were seen in lymph nodes, but they also were present in the heart, skeletal muscle, liver, testis, and adipose tissue. The incidences of hemangiomas and hemangiosarcomas were as follows:

	Control	Low Dose	High Dose
Hemangioma	2/20(10%)	8/50(16%)	5/49(10%)
Hemangiosarcoma	0/20(0%)	4/50(8%)	6/49(12%)
Total Number of Animals with Vascular Lesions	2/20(10%)	10/50(20%)	10/49(20%)

Based on the histopathologic examination, neoplasms of the liver were increased in female mice administered 2,4-diaminotoluene. Neoplasms of the liver, lung, and vascular system in the males, and neoplasms of the hematopoietic system in both the males and females may also be associated with administration of the test chemical.

D. Statistical Analyses of Results (Mice)

Tables F1 and F2 in Appendix F contain the statistical analyses of the incidences of those primary tumors that occurred in at least two animals of one group and at an incidence of at least 5% in one or more than one group.

In female mice, the result of the Cochran-Armitage test for positive dose-related trend in the incidence of hepatocellular carcinomas is significant (P = 0.002). The Fisher exact test shows that the incidence in either the low- or high-dose group is significantly higher than that in the control group (P = 0.007 and P = 0.001, respectively). The statistical conclusion is that the incidence of this liver tumor in female mice is associated with the administration of 2,4-diaminotoluene. The results of the statistical tests on the incidence of this tumor in male mice are not significant. No hepatocellular adenomas were reported in any group in either sex.

The result of the Cochran-Armitage test and that of the Fisher exact test comparing the high-dose group with the control group are not significant in the combined incidence of lymphoma and leukemia in female mice; however, the incidence in the low-dose group is significantly higher than that in the control group (P less than 0.001). Historical records of tests conducted at this laboratory indicate an incidence of animals with lymphomas or leukemias of 72/440 (16%), compared with incidences in the present bioassay of 2/19 (11%) in the control group, 29/47 (62%) in the low-dose group, and 11/46 (24%) in the high-dose group.

The Fisher exact comparison of the incidences of alveolar/bronchiolar carcinomas in low-dose and control groups of male mice indicates a P value of 0.039, which is above the 0.025 level required for significance when the Bonferroni inequality criterion is used for multiple comparison. The result of the Cochran-Armitage test and the incidence in the high-dose group are not significant.

In summary of the statistical evaluations, the incidence of hepatocellular carcinomas in the female mice is associated with the administration of the test chemical.

V. DISCUSSION

Mean body weights of dosed male and female rats and mice were lower than those of corresponding controls and were dose related except for the low-dose male mice for which mean body weights were only slightly lower than those of controls. Mortality was not dose related in either the male or female mice, but was dose related in both the male and female rats.

In the rats, hepatocellular carcinomas or neoplastic nodules occurred at incidences that were dose related in both the males (P = 0.014) and the females (P = 0.008). In direct comparisons of incidences of these tumors in control and dosed groups, the incidence in the high-dose male group had a P value of 0.026 (males: controls 0/20, low-dose 5/49, high-dose 10/50; females: controls 0/20, low-dose 0/50, high-dose 6/49). The significance of the occurrence of these tumors in both the male and female rats was supported by high incidences of associated nonneoplastic lesions of the liver in the dosed groups and by low incidences of liver tumors in historical-control male or female F344 rats at the same laboratory. In addition, early deaths may have reduced the incidences of the tumors in the high-dose groups. It is considered that the induction of a combination of hepatocellular carcinomas and neoplastic nodules in dosed rats was related to the administration of the test chemical.

In the female rats, carcinomas or adenomas of the mammary gland occurred at incidences that were dose related (P = 0.001) and in direct comparisons were higher in the dosed groups (P less than 0.001) than in the control group (controls 1/20, low-dose 38/50, high-dose 41/50).

In the male rats, fibromas of the subcutaneous tissue occurred at incidences that were dose related (P = 0.004) and in direct comparisons were higher in the dosed groups (P less than or equal to 0.020) than in the control group (controls 0/20, low-dose 15/30, high-dose 19/50).

Also, in the rats, hepatonephrotoxic lesions were observed in animals receiving 2,4-diaminotoluene. Chronic renal disease was more severe than that found in control animals, and in the liver, lesions ranged from scattered foci of lipidosis and cellular alteration to severe, diffuse toxic degenerative changes.

In the mice, hepatocellular carcinomas occurred in the females at incidences that were dose related (P = 0.002) and in direct comparisons were higher in the dosed groups (P less than or equal to 0.007) than in the control group (controls 0/19, low-dose 13/47, high-dose 18/46). In addition, lymphomas occurred at a significant incidence (P less than 0.001) in the low-dose female mice (controls 2/19, low-dose 29/47, high-dose 11/46). No tumors occurred at significant incidences in the male mice.

In previous long-term feeding studies (Ito et al., 1969), administration of 2,4-diaminotoluene to male Wistar rats for 36 weeks at 1,000 ppm induced hepatocellular carcinomas in all nine test animals, with multiple metastases in six of the animals and with numerous areas of nodular hyperplasia; rats administered 600 ppm had similar tumors in 6 of 11 test animals. No primary neoplasms were observed in organs other than the liver in the dosed animals, and the livers of the controls were essentially normal. Similar results were reported in 1-year studies at the DuPont Haskell Laboratories (Occupational Health and Safety Letter, 1975). In 2-year studies (Weisburger et al., in press), low incidences of liver tumors were observed in CD-1 (Sprague-Dawley) rats administered diets containing 2,4-diaminotoluene dihydrochloride at doses of 500 to 1,000 ppm for 4 months, then 250 or 500 ppm for 14 months, and in HaM/ICR mice administered the test chemical at doses of 500 or 1,000 ppm for 18 months.

A significant dose-related increase in the incidence of subcutaneous fibromas in the male rats was observed in this study. The occurrence of liver tumors in dosed male or female rats or female mice and of subcutaneous fibromas in dosed male rats of the present bioassay is in agreement with the results of the earlier studies. The occurrence of carcinomas or adenomas of the mammary gland in the female rats of the present bioassay was not observed in the earlier studies. When administered to rats of undefined strain and sex by 29 to 44 subcutaneous injections, 2,4-diaminotoluene was reported to induce local sarcomas (Umeda, 1955).

Under the conditions of this bioassay, 2,4-diaminotoluene was carcinogenic for male and female F344 rats, inducing hepatocellular carcinomas or neoplastic nodules in both males and females and carcinomas or adenomas of the mammary gland in females. The test chemical was also carcinogenic for B6C3F1 female mice, inducing hepatocellular carcinomas. The incidence of lymphomas in the female mice also suggested that these tumors may have been related to administration of the test chemical.

VI. BIBLIOGRAPHY

Armitage, P., Statistical Methods in Medical Research, John Wiley & Sons, Inc., New York, 1971, pp. 362-365.

Backus, J. K., Urethanes. In: Chemical and Process Technology Encyclopedia, Considine, D. M., ed., McGraw-Hill Book Co., New York, 1974, pp. 1121-1125.

Berenblum, I., ed., Carcinogenicity Testing: A Report of the Panel on Carcinogenicity of the Cancer Research Commission of UICC, Vol. 2, International Union Against Cancer, Geneva, 1969.

Cox, D. R., Regression models and life tables. J. R. Statist. Soc. B 34:187-220, 1972.

Cox, D. R., Analysis of Binary Data, Methuen & Co., Ltd., London, 1970, pp. 48-52.

Gart, J. J., The comparison of proportions: a review of significance tests, confidence limits and adjustments for stratification. Rev. Int. Stat. Inst. 39:148-169, 1971.

International Agency for Research on Cancer, IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man, Some Aromatic Amines and Related Nitro Compounds - Hair Dyes, Colouring Agents and Miscellaneous Industrial Chemicals, Vol. 16, World Health Organization, Geneva, 1978, pp. 83-95.

Ito, N., Hiasa, Y., Konishi, Y., and Marugami, M., The development of carcinoma in liver of rats treated with m-toluylenediamine and the synergistic and antagonistic effects with other chemicals. Cancer Res. 29:1137-1149, 1969.

Kaplan, E. L., and Meier, P., Nonparametric estimation from incomplete observations. J. Am. Statist. Assoc. 53:457-481, 1958.

Linhart, M. S., Cooper J. A., Martin, R. L., Page, N. P., and Peters, J. A., Carcinogenesis bioassay data system. Comp. and Biomed. Res. 7:230-248, 1974.

Miller, R. G., Jr., Simultaneous Statistical Inference, McGraw-Hill Book Co., New York, 1966, pp. 6-10.

Occupational Health and Safety Letter, 22 May 1975.

Saffiotti, U., Montesano, R., Sellakumar, A. R., Cefis, F., and Kauffman, D. G., Respiratory tract carcinogenesis in hamsters, induced by different numbers of administrations of benzo(a) pyrene and ferric oxide. Cancer Res. 32:1073-1081, 1972.

Society of Dyers and Colourists, Colour Index, Vol. 4, The Society of Dyers and Colourists, Bradford, England, 1971, p. 4850.

Squire, R. A., and Levitt, M. H., Report of a workshop on classification of specific hepatocellular lesions in rats. Cancer Res. 35:3214-3223, 1975.

Tarone, R. E., Tests for trend in life table analysis. Biometrika 62(3):679-682, 1975.

Umeda, M., Production of rat sarcoma by injections of propylene glycol solution of m-tolyulenediamine. Gann 46:597-603, 1955.

United States International Trade Commission, Synthetic Organic Chemicals - United States Production and Sales, 1976, USITC Publication 833, U. S. Government Printing Office, Washington, D. C., 1977a, p. 38.

United States International Trade Commission, Imports of Benzenoid Chemicals and Products, 1976, USITC Publication 828, Washington, D. C., 1977b, p. 27.

Wall, F. E., Bleaches, hair colorings, and dye removers. In: Cosmetics - Science and Technology, Vol. 2, Balsam, M. S., and Sagarin, E., eds., Wiley-Interscience, New York, 1972, pp. 306-309.

Weast, R. C., ed., Handbook of Chemistry and Physics, CRC Press, Cleveland, Ohio, 1974-1975.

Weisburger, E. K., Russfield, A. B., Homburger, F., Weisburger, J. H., Boger, E., Van Dongen, C. G., and Chu, K. C., Testing of 21 environmental aromatic amines or derivatives for long-term toxicity or carcinogenicity (in press). National Cancer Institute, Bethesda, Md.

APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS ADMINISTERED 2,4-DIAMINOTOLUENE IN THE DIET

TABLE A1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS ADMINISTERED 2,4–DIAMINOTOLUENE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
SQUAMOUS CELL CARCINOMA		2 (4%)	
*SUBCUT TISSUE	(20)	(50)	(50)
SQUAMOUS CELL CARCINOMA		2 (4%)	
SARCOMA, NOS		1 (2%)	
FIBROMA	1 (5%)	15 (30%)	19 (38%)
FIBROSARCOMA	1 (5%)	1 (2%)	3 (6%)
LIPOMA		3 (6%)	8 (16%)
FIBROADENOMA		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(20)	(50)	(50)
SQUAMOUS CELL CARCINOMA, METASTA		1 (2%)	
ALVEOLAR/BRONCHIOLAR ADENOMA		1 (2%)	1 (2%)
ALVEOLAR/BRONCHIOLAR CARCINOMA		4 (8%)	4 (8%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(50)
MONOCYTIC LEUKEMIA	1 (5%)	1 (2%)	1 (2%)
*SUBCUT TISSUE/AXILLA	(20)	(50)	(50)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (5%)		
#LYMPH NODE	(20)	(47)	(43)
SQUAMOUS CELL CARCINOMA, METASTA		1 (2%)	
#MESENTERIC L. NODE	(20)	(47)	(43)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
CIRCULATORY SYSTEM			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM			
#SUBLINGUAL GLAND	(20)	(45)	(48)
CARCINOMA-IN-SITU, NOS	1 (5%)		
#LIVER	(20)	(49)	(50)
NEOPLASTIC NODULE		2 (4%)	5 (10%)
HEPATOCELLULAR CARCINOMA		3 (6%)	6 (12%)
#PANCREAS	(19)	(42)	(44)
ACINAR-CELL ADENOMA	2 (11%)	10 (24%)	10 (23%)
FIBROMA		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(20)	(50)	(50)
TUBULAR-CELL ADENOMA		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(20)	(47)	(49)
CARCINOMA,NOS	2 (10%)	1 (2%)	
ADENOMA, NOS	2 (10%)	5 (11%)	1 (2%)
CHROMOPHOBE ADENOMA	4 (20%)	8 (17%)	8 (16%)
ACIDOPHIL ADENOMA		1 (2%)	
#ADRENAL	(20)	(49)	(50)
CORTICAL ADENOMA	1 (5%)	1 (2%)	2 (4%)
CORTICAL CARCINOMA	2 (10%)	2 (4%)	1 (2%)
PHEOCHROMOCYTOMA	1 (5%)	4 (8%)	8 (16%)
#THYROID	(20)	(44)	(47)
FOLLICULAR-CELL CARCINOMA		1 (2%)	1 (2%)
C-CELL ADENOMA	1 (5%)	6 (14%)	
C-CELL CARCINOMA		1 (2%)	
#PANCREATIC ISLETS	(19)	(42)	(44)
ISLET-CELL ADENOMA		2 (5%)	2 (5%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
ADENOMA, NOS		1 (2%)	1 (2%)

# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY

* NUMBER OF ANIMALS NECROPSIED

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
CYSTADENOCARCINOMA, NOS		1 (2%)	
FIBROMA		1 (2%)	
CARCINOSARCOMA		1 (2%)	
FIBROADENOMA		3 (6%)	4 (8%)
*PREPUTIAL GLAND	(20)	(50)	(50)
CARCINOMA,NOS		1 (2%)	
SQUAMOUS CELL CARCINOMA	1 (5%)	1 (2%)	
#PROSTATE	(19)	(41)	(42)
ADENOMA, NOS		1 (2%)	
#TESTIS	(20)	(50)	(50)
INTERSTITIAL-CELL TUMOR	15 (75%)	45 (90%)	44 (88%)
*EPIDIDYMIS	(20)	(50)	(50)
LIPOMA		1 (2%)	
NERVOUS SYSTEM			
#BRAIN	(20)	(49)	(50)
GLIOBLASTOMA MULTIFORME		1 (2%)	
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(20)	(50)	(50)
LIPOMA		1 (2%)	
MESOTHELIOMA, NOS		1 (2%)	3 (6%)
*EPICARDIUM	(20)	(50)	(50)
MESOTHELIOMA, NOS		1 (2%)	
*MESENTERY	(20)	(50)	(50)
LIPOMA			1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY

* NUMBER OF ANIMALS NECROPSIED

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
*TUNICA VAGINALIS	(20)	(50)	(50)
MESOTHELIOMA, NOS		4 (8%)	3 (6%)
MESOTHELIOMA, MALIGNANT			1 (2%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(50)	(50)
MESOTHELIOMA, NOS			1 (2%)
THORAX			
ALVEOLAR/BRONCHIOLAR CA, INVASIV		1	
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	3	17	10
MORIBUND SACRIFICE	1	11	40
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	16	22	
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	19	50	50
TOTAL PRIMARY TUMORS	36	145	138
TOTAL ANIMALS WITH BENIGN TUMORS	17	48	49
TOTAL BENIGN TUMORS	27	112	109
TOTAL ANIMALS WITH MALIGNANT TUMORS	7	20	13
TOTAL MALIGNANT TUMORS	9	25	17
TOTAL ANIMALS WITH SECONDARY TUMORS#		2	
TOTAL SECONDARY TUMORS		3	
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT		7	12
TOTAL UNCERTAIN TUMORS		8	12
TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

TABLE A2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS ADMINISTERED 2,4–DIAMINOTOLUENE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
PAPILLOMA, NOS		1 (2%)	
SQUAMOUS CELL CARCINOMA		3 (6%)	
*SUBCUT TISSUE	(20)	(50)	(50)
SARCOMA, NOS			1 (2%)
FIBROMA		4 (8%)	10 (20%)
FIBROSARCOMA		4 (8%)	
LIPOMA			1 (2%)
HEMANGIOMA		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(20)	(50)	(50)
HEPATOCELLULAR CARCINOMA, METAST			1 (2%)
ALVEOLAR/BRONCHIOLAR ADENOMA		1 (2%)	
ALVEOLAR/BRONCHIOLAR CARCINOMA	1 (5%)	3 (6%)	3 (6%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(50)
MONOCYTIC LEUKEMIA	3 (15%)	1 (2%)	
#SPLEEN	(19)	(48)	(48)
HEMANGIOSARCOMA		1 (2%)	
#LYMPH NODE	(20)	(49)	(49)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
#MESENTERIC L. NODE	(20)	(49)	(49)
HEPATOCELLULAR CARCINOMA, METAST			1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#THYMUS	(14)	(32)	(31)
THYMOMA		2 (6%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(20)	(50)	(49)
NEOPLASTIC NODULE			3 (6%)
HEPATOCELLULAR CARCINOMA			3 (6%)
#PANCREAS	(19)	(45)	(41)
ACINAR-CELL ADENOMA			3 (7%)
URINARY SYSTEM			
#KIDNEY	(20)	(49)	(49)
SARCOMA, NOS			1 (2%)
#URINARY BLADDER	(20)	(43)	(43)
PAPILLOMA, NOS			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(20)	(48)	(49)
CARCINOMA,NOS	1 (5%)	3 (6%)	1 (2%)
ADENOMA, NOS		4 (8%)	2 (4%)
ADENOCARCINOMA, NOS	1 (5%)		
CHROMOPHOBE ADENOMA	3 (15%)	14 (29%)	9 (18%)
ACIDOPHIL ADENOMA	2 (10%)	2 (4%)	2 (4%)
#ADRENAL	(20)	(49)	(49)
CORTICAL ADENOMA			3 (6%)
CORTICAL CARCINOMA		2 (4%)	
PHEOCHROMOCYTOMA	1 (5%)		1 (2%)
#THYROID	(20)	(49)	(48)
C-CELL ADENOMA		2 (4%)	1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY

* NUMBER OF ANIMALS NECROPSIED

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#PANCREATIC ISLETS	(19)	(45)	(41)
ISLET-CELL ADENOMA			1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
CARCINOMA,NOS		1 (2%)	3 (6%)
UNDIFFERENTIATED CARCINOMA		1 (2%)	
ADENOMA, NOS		10 (20%)	12 (24%)
ADENOCARCINOMA, NOS		4 (8%)	2 (4%)
PAPILLARY ADENOCARCINOMA		1 (2%)	1 (2%)
CYSTADENOMA, NOS		3 (6%)	5 (10%)
CYSTADENOCARCINOMA, NOS		2 (4%)	2 (4%)
PAPILLARY CYSTADENOMA, NOS			1 (2%)
CARCINOSARCOMA		1 (2%)	1 (2%)
FIBROADENOMA	1 (5%)	26 (52%)	29 (58%)
*VAGINA	(20)	(50)	(50)
LEIOMYOSARCOMA			1 (2%)
#UTERUS	(20)	(48)	(50)
LEIOMYOSARCOMA			1 (2%)
ENDOMETRIAL STROMAL POLYP	2 (10%)	9 (19%)	6 (12%)
ENDOMETRIAL STROMAL SARCOMA	1 (5%)		1 (2%)
#OVARY	(20)	(48)	(49)
GRANULOSA-CELL TUMOR		1 (2%)	
#MESOVARIUM	(20)	(48)	(49)
HEPATOCELLULAR CARCINOMA, METAST			1 (2%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
*RIB	(20)	(50)	(50)
OSTEOSARCOMA			1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
BODY CAVITIES			
*PLEURA	(20)	(50)	(50)
ALVEOLAR/BRONCHIOLAR CA, METASTA			1 (2%)
*MESENTERY	(20)	(50)	(50)
LIPOMA			1 (2%)
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	3	9	8
MORIBUND SACRIFICE	1	16	39
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	16	25	3
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	13	49	49
TOTAL PRIMARY TUMORS	16	108	113
TOTAL ANIMALS WITH BENIGN TUMORS	9	44	46
TOTAL BENIGN TUMORS	9	79	88
TOTAL ANIMALS WITH MALIGNANT TUMORS	7	20	19
TOTAL MALIGNANT TUMORS	7	28	22
TOTAL ANIMALS WITH SECONDARY TUMORS#			2
TOTAL SECONDARY TUMORS			4
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT		1	3
TOTAL UNCERTAIN TUMORS		1	3
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS

SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE ADMINISTERED 2,4-DIAMINOTOLUENE IN THE DIET

TABLE B1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE ADMINISTERED 2,4–DIAMINOTOLUENE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING			1
ANIMALS NECROPSIED	20	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	49
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(20)	(50)	(49)
LEIOMYOSARCOMA	1 (5%)		
NEURILEMOMA, MALIGNANT	1 (5%)		
RESPIRATORY SYSTEM			
#LUNG	(20)	(50)	(49)
ALVEOLAR/BRONCHIOLAR CARCINOMA		9 (18%)	6 (12%)
HEMANGIOMA			1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(49)
MALIGNANT LYMPHOMA, NOS		3 (6%)	2 (4%)
LEUKEMIA,NOS	1 (5%)		
#SPLEEN	(20)	(50)	(46)
HEMANGIOSARCOMA		1 (2%)	1 (2%)
MALIGNANT LYMPHOMA, NOS		5 (10%)	1 (2%)
#LYMPH NODE	(20)	(49)	(48)
HEMANGIOMA	1 (5%)	7 (14%)	2 (4%)
HEMANGIOSARCOMA		2 (4%)	1 (2%)
MALIGNANT LYMPHOMA, NOS	1 (5%)	5 (10%)	3 (6%)
#MESENTERIC L. NODE	(20)	(49)	(48)
MALIGNANT LYMPHOMA, NOS			1 (2%)
#LIVER	(20)	(50)	(49)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)
#PEYERS PATCH	(20)	(49)	(34)
MALIGNANT LYMPHOMA, NOS		2 (4%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY

* NUMBER OF ANIMALS NECROPSIED

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
CIRCULATORY SYSTEM			
#HEART	(20)	(50)	(48)
SARCOMA, NOS		1 (2%)	
HEMANGIOSARCOMA			1 (2%)
DIGESTIVE SYSTEM			
#LIVER	(20)	(50)	(49)
HEPATOCELLULAR CARCINOMA	5 (25%)	17 (34%)	13 (27%)
HEMANGIOMA	1 (5%)	1 (2%)	1 (2%)
HEMANGIOSARCOMA			2 (4%)
#JEJUNUM	(20)	(49)	(34)
ADENOCARCINOMA, NOS	1 (5%)		
ADENOMATOUS POLYP, NOS	1 (5%)		
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PANCREATIC ISLETS	(20)	(50)	(45)
ISLET-CELL CARCINOMA			1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(49)
UNDIFFERENTIATED CARCINOMA			1 (2%)
#TESTIS	(20)	(48)	(46)
HEMANGIOSARCOMA			1 (2%)
*EPIDIDYMIS	(20)	(50)	(49)
HEMANGIOSARCOMA			1 (2%)
NERVOUS SYSTEM			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(20)	(50)	(49)
PAPILLARY CARCINOMA		1 (2%)	1 (2%)
MUSCULOSKELETAL SYSTEM			
*SKELETAL MUSCLE	(20)	(50)	(49)
HEMANGIOSARCOMA, INVASIVE			1 (2%)
BODY CAVITIES			
*ABDOMINAL CAVITY	(20)	(50)	(49)
HEMANGIOSARCOMA			1 (2%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(50)	(49)
HEMANGIOSARCOMA, METASTATIC			1 (2%)
ADIPOSE TISSUE			
HEMANGIOMA			1
HEMANGIOSARCOMA		1	
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	1	4	6
MORIBUND SACRIFICE	1	1	
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	18	45	43
ANIMAL MISSING			1

@ INCLUDES AUTOLYZED ANIMALS

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	10	31	30
TOTAL PRIMARY TUMORS	13	55	43
TOTAL ANIMALS WITH BENIGN TUMORS	2	8	5
TOTAL BENIGN TUMORS	3	8	5
TOTAL ANIMALS WITH MALIGNANT TUMORS	10	28	28
TOTAL MALIGNANT TUMORS	10	47	38
TOTAL ANIMALS WITH SECONDARY TUMORS#			1
TOTAL SECONDARY TUMORS			2
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS

# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

TABLE B2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE ADMINISTERED 2,4–DIAMINOTOLUENE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING	1	3	4
ANIMALS NECROPSIED	19	47	46
ANIMALS EXAMINED HISTOPATHOLOGICALLY	19	47	46
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(19)	(47)	(46)
NEURILEMOMA, MALIGNANT	1 (5%)	1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(17)	(47)	(46)
ALVEOLAR/BRONCHIOLAR CARCINOMA		2 (4%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(19)	(47)	(46)
MALIGNANT LYMPHOMA, NOS		9 (19%)	3 (7%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)
LEUKEMIA,NOS	1 (5%)	18 (38%)	
LYMPHOCYTIC LEUKEMIA			1 (2%)
*BLOOD	(19)	(47)	(46)
LEUKEMIA,NOS		1 (2%)	
#BONE MARROW	(19)	(47)	(45)
HEMANGIOMA		1 (2%)	
#SPLEEN	(19)	(45)	(46)
HEMANGIOMA		1 (2%)	
HEMANGIOSARCOMA		2 (4%)	
#LYMPH NODE	(17)	(41)	(46)
HEMANGIOSARCOMA			1 (2%)
MALIGNANT LYMPHOMA, NOS		2 (5%)	4 (9%)
#MESENTERIC L. NODE	(17)	(41)	(46)
MALIGNANT LYMPHOMA, NOS	1 (6%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#LIVER	(19)	(47)	(46)
MALIGNANT LYMPHOMA, NOS			1 (2%)
LEUKEMIA,NOS			1 (2%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(17)	(45)	(45)
ADENOCARCINOMA, NOS		1 (2%)	
#LIVER	(19)	(47)	(46)
HEPATOCELLULAR CARCINOMA		13 (28%)	18 (39%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#ADRENAL	(18)	(47)	(46)
CORTICAL CARCINOMA		1 (2%)	
PHEOCHROMOCYTOMA		5 (11%)	
PHEOCHROMOCYTOMA, MALIGNANT		1 (2%)	
#THYROID	(17)	(44)	(44)
FOLLICULAR-CELL ADENOMA		1 (2%)	
FOLLICULAR-CELL CARCINOMA		2 (5%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(19)	(47)	(46)
CARCINOMA,NOS		1 (2%)	
ADENOCARCINOMA, NOS	1 (5%)		
*VAGINA	(19)	(47)	(46)
SQUAMOUS CELL CARCINOMA	1 (5%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#UTERUS	(18)	(45)	(45)
ADENOCARCINOMA, NOS		1 (2%)	1 (2%)
#OVARY	(18)	(44)	(44)
HEMANGIOMA		1 (2%)	
HEMANGIOSARCOMA			1 (2%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
*SKELETAL MUSCLE	(19)	(47)	(46)
HEMANGIOSARCOMA		1 (2%)	
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(19)	(47)	(46)
HEMANGIOSARCOMA			1 (2%)
SITE UNKNOWN			
LEIOMYOSARCOMA			1

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	3	7	4
MORIBUND SACRIFICE	1		2
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			1
TERMINAL SACRIFICE	15	40	39
ANIMAL MISSING	1	3	4

@ INCLUDES AUTOLYZED ANIMALS

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	5	40	26
TOTAL PRIMARY TUMORS	5	65	34
TOTAL ANIMALS WITH BENIGN TUMORS		7	
TOTAL BENIGN TUMORS		9	
TOTAL ANIMALS WITH MALIGNANT TUMORS	5	40	26
TOTAL MALIGNANT TUMORS	5	56	34
TOTAL ANIMALS WITH SECONDARY TUMORS#			
TOTAL SECONDARY TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS

# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS ADMINISTERED 2,4-DIAMINOTOLUENE IN THE DIET

TABLE C1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS ADMINISTERED 2,4–DIAMINOTOLUENE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
INFLAMMATION, NOS	1 (5%)		
RESPIRATORY SYSTEM			
#LUNG	(20)	(50)	(50)
INFLAMMATION, INTERSTITIAL	7 (35%)	1 (2%)	1 (2%)
HYPERPLASIA, ADENOMATOUS			2 (4%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(20)	(46)	(50)
HEMATOMA, ORGANIZED		1 (2%)	
FIBROSIS		3 (7%)	
FIBROSIS, FOCAL		1 (2%)	1 (2%)
#MANDIBULAR L. NODE	(20)	(47)	(43)
HYPERPLASIA, NOS	1 (5%)		
CIRCULATORY SYSTEM			
#HEART/ATRIUM	(20)	(50)	(50)
THROMBOSIS, NOS		3 (6%)	
#MYOCARDIUM	(20)	(50)	(50)
INFLAMMATION, CHRONIC			1 (2%)
FIBROSIS	18 (90%)	38 (76%)	35 (70%)
DEGENERATION, NOS	7 (35%)	16 (32%)	1 (2%)
CALCIFICATION, NOS		3 (6%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY

* NUMBER OF ANIMALS NECROPSIED

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(20)	(45)	(48)
HYPERPLASIA, NOS	1 (5%)	1 (2%)	
#PAROTID GLAND	(20)	(45)	(48)
FIBROSIS		2 (4%)	
CALCIFICATION, NOS		1 (2%)	
ATROPHY, NOS		1 (2%)	
#LIVER	(20)	(49)	(50)
HEMORRHAGE		1 (2%)	
INFLAMMATION, FOCAL		1 (2%)	
CHOLANGIOFIBROSIS	15 (75%)	17 (35%)	2 (4%)
DEGENERATION, CYSTIC		11 (22%)	2 (4%)
NECROSIS, FOCAL	1 (5%)	1 (2%)	
METAMORPHOSIS FATTY	1 (5%)	1 (2%)	
BASOPHILIC CYTO CHANGE		3 (6%)	
FOCAL CELLULAR CHANGE	2 (10%)	22 (45%)	36 (72%)
MEGALOCYTOSIS	1 (5%)		
HYPERPLASIA, DIFFUSE		1 (2%)	
ANGIECTASIS		1 (2%)	
#LIVER/CENTRILOBULAR	(20)	(49)	(50)
NECROSIS, DIFFUSE		1 (2%)	
METAMORPHOSIS FATTY	1 (5%)		
#LIVER/PERIPORTAL	(20)	(49)	(50)
INFLAMMATION, CHRONIC	1 (5%)		
#BILE DUCT	(20)	(49)	(50)
HYPERPLASIA, NOS	3 (15%)	1 (2%)	1 (2%)
#PANCREAS	(19)	(42)	(44)
HEMORRHAGIC CYST		1 (2%)	
INFLAMMATION, CHRONIC FOCAL	1 (5%)		
PERIARTERITIS		1 (2%)	
#PANCREATIC ACINUS	(19)	(42)	(44)
ATROPHY, FOCAL		1 (2%)	
#STOMACH	(19)	(48)	(49)
HYPERPLASIA, EPITHELIAL		1 (2%)	
#DUODENUM	(20)	(46)	(43)
INFLAMMATION, NECROTIZING		1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
URINARY SYSTEM			
#KIDNEY	(20)	(50)	(50)
PYELONEPHRITIS SUPPURATIVE		1 (2%)	1 (2%)
INFLAMMATION, CHRONIC	20 (100%)	50 (100%)	50 (100%)
#KIDNEY/TUBULE	(20)	(50)	(50)
NECROSIS, FOCAL	1 (5%)		
#URINARY BLADDER	(20)	(36)	(39)
HEMORRHAGE	1 (5%)		
INFLAMMATION, CHRONIC FOCAL		1 (3%)	
HYPERPLASIA, EPITHELIAL		1 (3%)	
ENDOCRINE SYSTEM			
#ANTERIOR PITUITARY	(20)	(47)	(49)
CYST, NOS	1 (5%)		
#ADRENAL	(20)	(49)	(50)
FIBROSIS, FOCAL	1 (5%)		
INFARCT HEMORRHAGIC		1 (2%)	1 (2%)
ANGIECTASIS			1 (2%)
#ADRENAL CORTEX	(20)	(49)	(50)
CYST, NOS	1 (5%)		
#ADRENAL MEDULLA	(20)	(49)	(50)
HYPERPLASIA, NOS		2 (4%)	3 (6%)
#THYROID	(20)	(44)	(47)
HYPERPLASIA, C-CELL		1 (2%)	2 (4%)
#THYROID FOLLICLE	(20)	(44)	(47)
ATROPHY, NOS			2 (4%)
HYPERPLASIA, PAPILLARY			1 (2%)
#PARATHYROID	(16)	(42)	(40)
HYPERPLASIA, NOS		8 (19%)	8 (20%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
GALACTOCELE	1 (5%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
HYPERPLASIA, NOS		13 (26%)	6 (12%)
HYPERPLASIA, FOCAL		1 (2%)	
HYPERPLASIA, CYSTIC			1 (2%)
LACTATION	1 (5%)		2 (4%)
#PROSTATE	(19)	(41)	(42)
ABSCESS, NOS			1 (2%)
INFLAMMATION, CHRONIC		4 (10%)	
FIBROSIS	1 (5%)	3 (7%)	
HYPERPLASIA, NOS		2 (5%)	
HYPERPLASIA, FOCAL		1 (2%)	
HYPERPLASIA, PAPILLARY	1 (5%)		
METAPLASIA, SQUAMOUS		1 (2%)	
*SEMINAL VESICLE	(20)	(50)	(50)
ABSCESS, NOS		1 (2%)	
#TESTIS	(20)	(50)	(50)
ABSCESS, NOS		1 (2%)	1 (2%)
ATROPHY, NOS			1 (2%)
HYPERPLASIA, INTERSTITIAL CELL	2 (10%)		
*EPIDIDYMIS	(20)	(50)	(50)
GRANULOMA, SPERMATIC		1 (2%)	
NERVOUS SYSTEM			
#BRAIN/MENINGES	(20)	(49)	(50)
INFLAMMATION, SUPPURATIVE		1 (2%)	
INFARCT HEMORRHAGIC		1 (2%)	
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS ADMINISTERED 2,4–DIAMINOTOLUENE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
EPIDERMAL INCLUSION CYST			2 (4%)
*SUBCUT TISSUE	(20)	(50)	(50)
GRANULOMA, FOREIGN BODY	1 (5%)		
HYPERPLASIA, NOS			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(20)	(50)	(50)
INFLAMMATION, INTERSTITIAL	6 (30%)	9 (18%)	8 (16%)
BRONCHOPNEUMONIA NECROTIZING		1 (2%)	
INFLAMMATION, FOCAL GRANULOMATOU			1 (2%)
HYPERPLASIA, ADENOMATOUS			2 (4%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(20)	(50)	(47)
HYPERPLASIA, NOS	1 (5%)		
MEGAKARYOCYTOSIS	1 (5%)		
HYPERPLASIA, HEMATOPOIETIC		1 (2%)	
#SPLEEN	(19)	(48)	(48)
HEMATOPOIESIS		1 (2%)	
#LYMPH NODE	(20)	(49)	(49)
HYPERPLASIA, NOS		2 (4%)	
PLASMACYTOSIS		1 (2%)	
#THYMUS	(14)	(32)	(31)
EPIDERMAL INCLUSION CYST			1 (3%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
HYPERPLASIA, EPITHELIAL			1 (3%)
CIRCULATORY SYSTEM			
#HEART	(20)	(50)	(50)
EMBOLUS, SEPTIC	1 (5%)		
#MYOCARDIUM	(20)	(50)	(50)
THROMBOSIS, NOS	1 (5%)		
INFLAMMATION, CHRONIC			1 (2%)
INFLAMMATION, CHRONIC FOCAL		1 (2%)	1 (2%)
FIBROSIS	12 (60%)	24 (48%)	22 (44%)
DEGENERATION, NOS		12 (24%)	
DIGESTIVE SYSTEM			
#PAROTID GLAND	(20)	(49)	(49)
FIBROSIS		1 (2%)	
ATROPHY, NOS		1 (2%)	
#LIVER	(20)	(50)	(49)
INFLAMMATION, NECROTIZING		3 (6%)	
INFLAMMATION, CHRONIC FOCAL	1 (5%)		
CHOLANGIOFIBROSIS	9 (45%)	6 (12%)	3 (6%)
HEPATITIS, TOXIC	1 (5%)	1 (2%)	
NECROSIS, FOCAL		2 (4%)	
LIPOIDOSIS	1 (5%)		
BASOPHILIC CYTO CHANGE		2 (4%)	4 (8%)
FOCAL CELLULAR CHANGE	1 (5%)	21 (42%)	38 (78%)
ANGIECTASIS			4 (8%)
#BILE DUCT	(20)	(50)	(49)
INFLAMMATION, CHRONIC		1 (2%)	
HYPERPLASIA, NOS	5 (25%)	10 (20%)	
#PANCREAS	(19)	(45)	(41)
FIBROSIS		1 (2%)	
ATROPHY, FOCAL		1 (2%)	
#STOMACH	(20)	(48)	(48)
EPIDERMAL INCLUSION CYST		1 (2%)	
ULCER, NOS		1 (2%)	
ULCER, ACUTE	1 (5%)		

# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#DUODENUM	(20)	(50)	(49)
INFLAMMATION, CHRONIC NECROTIZIN		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(20)	(49)	(49)
CYST, NOS		1 (2%)	
INFLAMMATION, CHRONIC	15 (75%)	45 (92%)	48 (98%)
FIBROSIS			1 (2%)
DEGENERATION, HYALINE		1 (2%)	
NEPHROSIS, NOS	1 (5%)		
INFARCT, FOCAL	1 (5%)		
#KIDNEY/TUBULE	(20)	(49)	(49)
DEGENERATION, NOS		1 (2%)	
DEGENERATION, HYALINE			1 (2%)
NECROSIS, NOS		1 (2%)	
NECROSIS, FOCAL	1 (5%)		
#URINARY BLADDER	(20)	(43)	(43)
INFLAMMATION, CHRONIC FOCAL		4 (9%)	20 (47%)
HYPERPLASIA, EPITHELIAL	1 (5%)	1 (2%)	
POLYP	1 (5%)		
ENDOCRINE SYSTEM			
#PITUITARY	(20)	(48)	(49)
CYST, NOS	1 (5%)		
#ADRENAL	(20)	(49)	(49)
DEGENERATION, CYSTIC	5 (25%)	5 (10%)	4 (8%)
NECROSIS, HEMORRHAGIC		1 (2%)	
METAMORPHOSIS FATTY		3 (6%)	
#ADRENAL CORTEX	(20)	(49)	(49)
DEGENERATION, CYSTIC	1 (5%)		
HYPERPLASIA, NOS	1 (5%)		
#ADRENAL MEDULLA	(20)	(49)	(49)
HYPERPLASIA, NOS	1 (5%)	1 (2%)	
#THYROID	(20)	(49)	(48)
HYPERPLASIA, C-CELL	1 (5%)	1 (2%)	1 (2%)

# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
HYPERPLASIA, CYSTIC		1 (2%)	2 (4%)
#UTERUS/ENDOMETRIUM	(20)	(48)	(50)
FIBROSIS	19 (95%)	42 (88%)	43 (86%)
HYPERPLASIA, CYSTIC			1 (2%)
#OVARY	(20)	(48)	(49)
CYST, NOS			1 (2%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(20)	(50)	(50)
LIPOGRANULOMA			1 (2%)
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY

* NUMBER OF ANIMALS NECROPSIED

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE ADMINISTERED 2,4-DIAMINOTOLUENE IN THE DIET

TABLE D1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE ADMINISTERED 2,4–DIAMINOTOLUENE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING			1
ANIMALS NECROPSIED	20	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	49
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(20)	(50)	(49)
FIBROSIS			5 (10%)
HYPERPLASTIC NODULE		1 (2%)	
HYPERPLASIA, ALVEOLAR EPITHELIUM		1 (2%)	1 (2%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(20)	(47)	(49)
FIBROSIS, FOCAL			1 (2%)
#SPLEEN	(20)	(50)	(46)
HYPERPLASIA, NOS		2 (4%)	
#LYMPH NODE	(20)	(49)	(48)
HYPERPLASIA, NOS		8 (16%)	1 (2%)
#THYMUS	(15)	(35)	(17)
HYPERPLASIA, NOS		1 (3%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(20)	(50)	(49)
DEGENERATION, HYDROPIC			1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
INFARCT, NOS			1 (2%)
METAMORPHOSIS FATTY			1 (2%)
ATYPIA, NOS			1 (2%)
HYPERPLASTIC NODULE**		6 (12%)	4 (8%)
HYPERPLASIA, NOS		11 (22%)	26 (53%)
HYPERPLASIA, DIFFUSE		8 (16%)	4 (8%)
#DUODENAL GLAND	(20)	(49)	(34)
HYPERPLASIA, NOS		1 (2%)	
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(19)	(49)	(45)
NODULE		1 (2%)	
#ADRENAL	(20)	(50)	(47)
NODULE		3 (6%)	3 (6%)
#ADRENAL CORTEX	(20)	(50)	(47)
NODULE		2 (4%)	1 (2%)
REPRODUCTIVE SYSTEM			
NONE			
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			

# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

** NODULAR HYPERPLASIA

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	10	2	1
ANIMAL MISSING/NO NECROPSY			1
AUTO/NECROPSY/HISTO PERF			1

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE ADMINISTERED 2,4–DIAMINOTOLUENE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING	1	3	4
ANIMALS NECROPSIED	19	47	46
ANIMALS EXAMINED HISTOPATHOLOGICALLY	19	47	46
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(17)	(47)	(46)
HYPERPLASTIC NODULE		1 (2%)	1 (2%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(19)	(47)	(45)
HYPERPLASIA, NOS		2 (4%)	
#THYMUS	(8)	(28)	(33)
ATROPHY, NOS			1 (3%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(19)	(47)	(46)
NECROSIS, FOCAL		1 (2%)	1 (2%)
INFARCT, NOS			1 (2%)
LIPOIDOSIS			1 (2%)
HYPERPLASTIC NODULE**		9 (19%)	22 (48%)
HYPERPLASIA, NOS		8 (17%)	5 (11%)
HYPERPLASIA, DIFFUSE		13 (28%)	
#PANCREATIC ACINUS	(16)	(45)	(45)
HYPERPLASIA, NOS		1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

** NODULAR HYPERPLASIA

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#STOMACH	(19)	(46)	(43)
EROSION			1 (2%)
URINARY SYSTEM			
#KIDNEY/TUBULE	(18)	(46)	(46)
DILATATION, NOS			1 (2%)
ENDOCRINE SYSTEM			
#ADRENAL	(18)	(47)	(46)
NODULE			1 (2%)
#ADRENAL CORTEX	(18)	(47)	(46)
NODULE		1 (2%)	
#PANCREATIC ISLETS	(16)	(45)	(45)
HYPERPLASIA, NOS		1 (2%)	
REPRODUCTIVE SYSTEM			
#UTERUS	(18)	(45)	(45)
POLYP			1 (2%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	13	1	7
ANIMAL MISSING/NO NECROPSY	1	3	4
AUTO/NECROPSY/HISTO PERF	1		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

APPENDIX E

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN RATS ADMINISTERED 2,4-DIAMINOTOLUENE IN THE DIET

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered 2,4-Diaminotoluene in the Diet (a)

Topography: Morphology	Matched Control	Low Dose	High Dose
Integumentary System: Squamous-cell Carcinoma (b)	0/20 (0)	4/50 (8)	0/50 (0)
P Values (c,d)	N.S.	N.S.	--
Departure from Linear Trend (e)	P = 0.024		
Relative Risk (f)		Infinite	--
Lower Limit		0.386	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	76	--
Integumentary System: Fibroma of the Subcutaneous Tissue (b)	1/20 (5)	15/50 (30)	19/50 (38)
P Values (c,d)	P = 0.009	P = 0.020	P = 0.004
Relative Risk (f)		6.000	7.600
Lower Limit		1.048	1.377
Upper Limit		245.704	305.928
Weeks to First Observed Tumor	103	71	49

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered 2,4-Diaminotoluene in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Integumentary System: Fibrosarcoma of the Subcutaneous Tissue (b)	1/20 (5)	1/50 (2)	3/50 (6)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.400	1.200
Lower Limit		0.005	0.106
Upper Limit		30.802	61.724
Weeks to First Observed Tumor	103	103	70
Integumentary System: Lipoma of the Subcutaneous Tissue (b)	0/20 (0)	3/50 (6)	8/50 (16)
P Values (c,d)	P = 0.017	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.250	0.952
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	76	71

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered 2,4-Diaminotoluene in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Carcinoma (b)	0/20 (0)	4/50 (8)	4/50 (8)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.386	0.386
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	76	78
Lung: Alveolar/Bronchiolar Carcinoma or Adenoma (b)	0/20 (0)	5/50 (10)	5/50 (10)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.525	0.525
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	76	78

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered 2,4-Diaminotoluene in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System: Lymphoma or Leukemia (b)	2/20 (10)	2/50 (4)	1/50 (2)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.400	0.200
Lower Limit		0.032	0.004
Upper Limit		5.277	3.681
Weeks to First Observed Tumor	103	85	62
Liver: Hepatocellular Carcinoma (b)	0/20 (0)	3/49 (6)	6/50 (12)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.255	0.667
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	76	57

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered 2,4-Diaminotoluene in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Liver: Hepatocellular Carcinoma or Neoplastic Nodule (b)	0/20 (0)	5/49 (10)	10/50 (20)
P Values (c,d)	P = 0.014	N.S.	P = 0.026
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.536	1.240
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	76	53
Pancreas: Acinar-cell Adenoma (b)	2/19 (11)	10/42 (24)	10/44 (23)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		2.262	2.159
Lower Limit		0.558	0.532
Upper Limit		19.947	19.089
Weeks to First Observed Tumor	103	89	71

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered 2,4-Diaminotoluene in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Pituitary: Carcinoma, NOS (b)	2/20 (10)	1/47 (2)	0/49 (0)
P Values (c,d)	P = 0.037 (N)	N.S.	N.S.
Relative Risk (f)		0.213	0.000
Lower Limit		0.004	0.000
Upper Limit		3.909	1.372
Weeks to First Observed Tumor	103	103	--
Pituitary: Adenoma, NOS (b)	2/20 (10)	5/47 (11)	1/49 (2)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.064	0.204
Lower Limit		0.196	0.004
Upper Limit		10.623	3.754
Weeks to First Observed Tumor	103	84	71

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered 2,4-Diaminotoluene in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Pituitary: Chromophobe Adenoma (b)	4/20 (20)	8/47 (17)	8/49 (16)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.851	0.816
Lower Limit		0.266	0.255
Upper Limit		3.528	3.392
Weeks to First Observed Tumor	103	72	63
Adrenal: Cortical Carcinoma (b)	2/20 (10)	2/49 (4)	1/50 (2)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.408	0.200
Lower Limit		0.032	0.004
Upper Limit		5.381	3.681
Weeks to First Observed Tumor	80	102	69

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered 2,4-Diaminotoluene in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Adrenal: Cortical Carcinoma or Adenoma (b)	3/20 (15)	3/49 (6)	3/50 (6)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.408	0.400
Lower Limit		0.061	0.060
Upper Limit		2.857	2.802
Weeks to First Observed Tumor	83	102	69
Adrenal: Pheochromocytoma (b)	1/20 (5)	4/49 (8)	8/50 (16)
P.Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.633	3.200
Lower Limit		0.179	0.482
Upper Limit		78.704	138.771
Weeks to First Observed Tumor	103	78	76

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered 2,4-Diaminotoluene in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Thyroid: C-cell Carcinoma or Adenoma (b)	1/20 (5)	7/44 (16)	0/47 (0)
P Values (c,d)	N.S.	N.S.	N.S.
Departure From Linear Trend (e)	P = 0.014		
Relative Risk (f)		3.182	0.000
Lower Limit		0.459	0.000
Upper Limit		139.691	7.942
Weeks to First Observed Tumor	103	71	--
Pancreatic Islets: Islet-cell Adenoma (b)	0/19 (0)	2/42 (5)	2/44 (5)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.139	0.133
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	103	78

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered 2,4-Diaminotoluene in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Mammary Gland: All Adenoma (b)	0/20 (0)	4/50 (8)	5/50 (10)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.386	0.525
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	95	49
Mammary Gland: Adenoma or Carcinoma (b)	0/20 (0)	5/50 (10)	5/50 (10)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.525	0.525
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	103	49

NIH LIBR

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered 2,4-Diaminotoluene in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Testis: Interstitial-cell Tumor (b)	15/20 (75)	45/50 (90)	44/50 (88)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.200	1.173
Lower Limit		0.935	0.911
Upper Limit		1.582	1.585
Weeks to First Observed Tumor	71	62	49
Abdominal Cavity: Mesothelioma, NOS (b)	0/20 (0)	1/50 (2)	3/50 (6)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.022	0.250
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	---	103	57

NIH LIBR

Table El. Analyses of the Incidence of Primary Tumors in Male Rats Administered 2,4-Diaminotoluene in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Tunica Vaginalis: Mesothelioma, NOS (b)	0/20 (0)	4/50 (8)	3/50 (6)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.386	0.250
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	98	78
All Sites: Mesothelioma (b)	0/20 (0)	5/50 (10)	8/50 (16)
P Values	P = 0.042	N.S.	N.S.
Relative Risk		Infinite	Infinite
Lower Limit		0.525	0.952
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	98	57

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered 2,4-Diaminotoluene in the Diet (a)

(continued)

(a) Dosed groups received time-weighted average doses of 79 or 176 ppm.

(b) Number of tumor-bearing animals/number of animals examined at site (percent).

(c) Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P is less than 0.05; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the matched-control group when P is less than 0.05; otherwise, not significant (N.S.) is indicated.

(d) A negative trend (N) indicates a lower incidence in a dosed group than in the control group.

(e) The probability level for departure from linear trend is given when P is less than 0.05 for any comparison.

(f) The 95% confidence interval of the relative risk between each dosed group and the control group.

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered 2,4-Diaminotoluene in the Diet (a)

Topography: Morphology	Matched Control	Low Dose	High Dose
Integumentary System: Squamous-cell Carcinoma of the Skin (b)	0/20 (0)	3/50 (6)	0/50 (0)
P Values (c,d)	N.S.	N.S.	--
Relative Risk (f)		Infinite	--
Lower Limit		0.250	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	84	--
Integumentary System: Fibroma of the Subcutaneous Tissue (b)	0/20 (0)	4/50 (8)	10/50 (20)
P Values (c,d)	P = 0.009	N.S.	P = 0.026
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.386	1.240
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	84	70

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered 2,4-Diaminotoluene in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Integumentary System: Fibrosarcoma of the Subcutaneous Tissue (b)	0/20 (0)	4/50 (8)	0/50 (0)
P Values (c,d)	N.S.	N.S.	--
Departure from Linear Trend (e)	P = 0.023		
Relative Risk (f)		Infinite	--
Lower Limit		0.386	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	41	--
Lung: Alveolar/Bronchiolar Carcinoma (b)	1/20 (5)	3/50 (6)	3/50 (6)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.200	1.200
Lower Limit		0.106	0.106
Upper Limit		61.724	61.724
Weeks to First Observed Tumor	103	84	84

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered 2,4-Diaminotoluene in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Carcinoma or Adenoma (b)	1/20 (5)	4/50 (8)	3/50 (6)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.600	1.200
Lower Limit		0.175	0.106
Upper Limit		77.169	61.724
Weeks to First Observed Tumor	103	84	84
Hematopoietic System: Lymphoma or Leukemia (b)	3/20 (15)	2/50 (4)	0/50 (0)
P Values (c,d)	P = 0.010 (N)	N.S.	P = 0.021 (N)
Relative Risk (f)		0.267	0.000
Lower Limit		0.024	0.000
Upper Limit		2.190	0.659
Weeks to First Observed Tumor	99	84	--

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered 2,4-Diaminotoluene in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Thymus: Thymoma (b)	0/14 (0)	2/32 (6)	0/31 (0)
P Values (c,d)	N.S.	N.S.	--
Relative Risk (f)		Infinite	--
Lower Limit		0.138	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	84	--
Liver: Hepatocellular Carcinoma (b)	0/20 (0)	0/50 (0)	3/49 (6)
P Values (c,d)	N.S.	--	N.S.
Relative Risk (f)		--	Infinite
Lower Limit		--	0.255
Upper Limit		--	Infinite
Weeks to First Observed Tumor	--	--	80

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered 2,4-Diaminotoluene in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Liver: Hepatocellular Carcinoma or Neoplastic Nodule (b)	0/20 (0)	0/50 (0)	6/49 (12)
P Values (c,d)	P = 0.008	--	N.S.
Relative Risk (f)		--	Infinite
Lower Limit		--	0.680
Upper Limit		--	Infinite
Weeks to First Observed Tumor	--	--	80
Pancreas: Acinar-cell Adenoma (b)	0/19 (0)	0/45 (0)	3/41 (7)
P Values (c,d)	N.S.	--	N.S.
Relative Risk (f)		--	Infinite
Lower Limit		--	0.291
Upper Limit		--	Infinite
Weeks to First Observed Tumor	--	--	84

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered 2,4-Diaminotoluene in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Pituitary: Carcinoma, NOS (b)	1/20 (5)	3/48 (6)	1/49 (2)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.250	0.408
Lower Limit		0.110	0.005
Upper Limit		64.251	31.413
Weeks to First Observed Tumor	103	89	84
Pituitary: Adenoma, NOS (b)	0/20 (0)	4/48 (8)	2/49 (4)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.402	0.125
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	84	84

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered 2,4-Diaminotoluene in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Pituitary: Chromophobe Adenoma (b)	3/20 (15)	14/48 (29)	9/49 (18)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.944	1.224
Lower Limit		0.635	0.354
Upper Limit		9.723	6.533
Weeks to First Observed Tumor	88	76	78
Pituitary: Acidophil Adenoma (b)	2/20 (10)	2/48 (4)	2/49 (4)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.417	0.408
Lower Limit		0.033	0.032
Upper Limit		5.490	5.381
Weeks to First Observed Tumor	94	103	84

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered 2,4-Diaminotoluene in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Adrenal: Cortical Carcinoma or Adenoma (b)	0/20 (0)	2/49 (4)	3/49 (6)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.125	0.255
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	103	84
Mammary Gland: All Adenoma (b)	1/20 (5)	34/50 (68)	38/50 (76)
P Values (c,d)	P less than 0.001	P less than 0.001	P less than 0.001
Departure from Linear Trend (e)	P = 0.002		
Relative Risk (f)		13.600	15.200
Lower Limit		2.656	3.023
Upper Limit		519.231	565.873
Weeks to First Observed Tumor	103	84	76

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered 2,4-Diaminotoluene in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Mammary Gland: All Carcinoma (b)	0/20 (0)	9/50 (18)	8/50 (16)
P Values (c,d)	N.S.	P = 0.039	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		1.096	0.952
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	40	84
Mammary Gland: Adenoma or Carcinoma (b)	1/20 (5)	38/50 (76)	41/50 (82)
P Values (c,d)	P less than 0.001	P less than 0.001	P less than 0.001
Departure from Linear Trend (e)	P less than 0.001		
Relative Risk (f)		15.200	16.400
Lower Limit		3.023	3.320
Upper Limit		565.873	591.602
Weeks to First Observed Tumor	103	40	76

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered 2,4-Diaminotoluene in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Uterus: Endometrial Stromal Polyp (b)	2/20 (10)	9/48 (19)	6/50 (12)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.875	1.200
Lower Limit		0.444	0.243
Upper Limit		16.902	11.574
Weeks to First Observed Tumor	99	86	76

(a) Dosed groups received time-weighted average doses of 79 or 171 ppm.

(b) Number of tumor-bearing animals/number of animals examined at site (percent).

(c) Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P is less than 0.05; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the matched-control group when P is less than 0.05; otherwise, not significant (N.S.) is indicated.

(d) A negative trend (N) indicates a lower incidence in a dosed group than in the control group.

(e) The probability level for departure from linear trend is given when P is less than 0.05 for any comparison.

(f) The 95% confidence interval of the relative risk between each dosed group and the control group.

APPENDIX F

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN MICE ADMINISTERED 2,4-DIAMINOTOLUENE IN THE DIET

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered 2,4-Diaminotoluene in the Diet (a)

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Carcinoma (b)	0/20 (0)	9/50 (18)	6/49 (12)
P Values (c,d)	N.S.	P = 0.039	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		1.096	0.680
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	101	101
Hematopoietic System: Lymphoma or Leukemia (b)	2/20 (10)	15/50 (30)	8/49 (16)
P Values (c,d)	N.S.	N.S.	N.S.
Departure from Linear Trend (e)	P = 0.033		
Relative Risk (f)		3.000	1.633
Lower Limit		0.805	0.371
Upper Limit		25.510	14.987
Weeks to First Observed Tumor	84	91	60

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered 2,4-Diaminotoluene in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
All Sites: Hemangiosarcoma (b)	0/20 (0)	4/50 (8)	6/49 (12)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.386	0.680
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	91	101
All Sites: Hemangioma (b)	2/20 (10)	8/50 (16)	5/49 (10)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.600	1.020
Lower Limit		0.364	0.188
Upper Limit		14.699	10.204
Weeks to First Observed Tumor	101	101	101

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered 2,4-Diaminotoluene in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
All Sites: Hemangiosarcoma or Hemangioma (b)	2/20 (10)	10/50 (20)	10/49 (20)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		2.000	2.041
Lower Limit		0.488	0.498
Upper Limit		17.808	18.154
Weeks to First Observed Tumor	101	91	101
Liver: Hepatocellular Carcinoma (b)	5/20 (25)	17/50 (34)	13/49 (27)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.360	1.061
Lower Limit		0.580	0.425
Upper Limit		4.195	3.404
Weeks to First Observed Tumor	101	85	84

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered 2,4-Diaminotoluene in the Diet (a)

(continued)

(a) Dosed groups received 100 or 200 ppm.

(b) Number of tumor-bearing animals/number of animals examined at site (percent).

(c) Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P is less than 0.05; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the matched-control group when P is less than 0.05; otherwise, not significant (N.S.) is indicated.

(d) A negative trend (N) indicates a lower incidence in a dosed group than in the control group.

(e) The probability level for departure from linear trend is given when P is less than 0.05 for any comparison.

(f) The 95% confidence interval of the relative risk between each dosed group and the control group.

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Administered 2,4-Diaminotoluene in the Diet (a)

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System: Lymphoma or Leukemia (b)	2/19 (11)	29/47 (62)	11/46 (24)
P Values (c,d)	N.S.	P less than 0.001	N.S.
Departure from Linear Trend (e)	P less than 0.001		
Relative Risk (f)		5.862	2.272
Lower Limit		1.761	0.573
Upper Limit		45.960	19.887
Weeks to First Observed Tumor	101	81	71
All Sites: Hemangioma (b)	0/19 (0)	3/47 (6)	0/46 (0)
P Values (c,d)	N.S.	N.S.	--
Departure from Linear Trend (e)	P = 0.047		
Relative Risk (f)		Infinite	--
Lower Limit		0.254	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	101	--

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Administered 2,4-Diaminotoluene in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
All Sites: Hemangiosarcoma (b)	0/19 (0)	3/47 (6)	3/46 (7)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.254	0.259
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	96	96
All Sites: Hemangioma or Hemangiosarcoma (b)	0/19 (0)	5/47 (11)	3/46 (7)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.533	0.259
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	96	96

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Administered 2,4-Diaminotoluene in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Liver: Hepatocellular Carcinoma (b)	0/19 (0)	13/47 (28)	18/46 (39)
P Values (c,d)	P = 0.002	P = 0.007	P = 0.001
Relative Risk (f)		Infinite	Infinite
Lower Limit		1.699	2.493
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	101	71
Adrenal: Pheochromocytoma (b)	0/18 (0)	6/47 (13)	0/46 (0)
P Values (c,d)	N.S.	N.S.	--
Departure from Linear Trend (e)	P = 0.006		
Relative Risk (f)		Infinite	--
Lower Limit		0.643	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	101	--

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Administered 2,4-Diaminotoluene in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Thyroid: Follicular-cell Carcinoma (b)	0/17 (0)	2/44 (5)	0/44 (0)
P Values (c,d)	N.S.	N.S.	--
Relative Risk (f)		Infinite	--
Lower Limit		0.120	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	101	--
Thyroid: Follicular-cell Carcinoma or Adenoma (b)	0/17 (0)	3/44 (7)	0/44 (0)
P Values (c,d)	N.S.	N.S.	--
Departure from Linear Trend (e)	P = 0.048		
Relative Risk (f)		Infinite	--
Lower Limit		0.244	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	101	--

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Administered 2,4-Diaminotoluene in the Diet (a)

(continued)

(a) Dosed groups received 100 or 200 ppm.

(b) Number of tumor-bearing animals/number of animals examined at site (percent).

(c) Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P is less than 0.05; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the matched-control group when P is less than 0.05; otherwise, not significant (N.S.) is indicated.

(d) A negative trend (N) indicates a lower incidence in a dosed group than in the control group.

(e) The probability level for departure from linear trend is given when P is less than 0.05 for any comparison.

(f) The 95% confidence interval of the relative risk between each dosed group and the control group.

Review of the Bioassay of 2,4-Diaminotoluene* for Carcinogenicity by the Data Evaluation/Risk Assessment Subgroup of the Clearinghouse on Environmental Carcinogens

December 13, 1978

The Clearinghouse on Environmental Carcinogens was established in May, 1976, in compliance with DHEW Committee Regulations and the Provisions of the Federal Advisory Committee Act. The purpose of the Clearinghouse is to advise the Director of the National Cancer Institute on the Institute's bioassay program to identify and evaluate chemical carcinogens in the environment to which humans may be exposed. The members of the Clearinghouse have been drawn from academia, industry, organized labor, public interest groups, and State health officials. Members have been selected on the basis of their experience in carcinogenesis or related fields and, collectively, provide expertise in chemistry, biochemistry, biostatistics, toxicology, pathology, and epidemiology. Representatives of various Governmental agencies participate as ad hoc members. The Data Evaluation/Risk Assessment Subgroup of the Clearinghouse is charged with the responsibility of providing a peer review of reports prepared on NCI-sponsored bioassays of chemicals studied for carcinogenicity. It is in this context that the below critique is given on the bioassay of 2,4-Diaminotoluene.

The primary reviewer for the report on the bioassay of 2,4-Diaminotoluene said that the compound was carcinogenic in both sexes of treated rats and in treated female mice. Increased incidences of hemangiosarcomas and hemangiomas were also observed in treated male mice, but they were not statistically significant. After a brief description of the experimental design, he mentioned several other tumor types found at an increased but not statistically significant incidence. Because of the wide variety of neoplasms associated with treatment, the reviewer opined that 2,4-Diaminotoluene was a "potent" carcinogen and poses a potential risk to humans.

The secondary reviewer emphasized the potential hepato-nephrotic hazard of 2,4-Diaminotoluene. He also pointed out the increased incidence of lung tumors among the treated animals.

It was moved that the report on the bioassay of 2,4-Diaminotoluene be accepted as written. The motion was seconded and approved without objection.

Clearinghouse Members Present:

Arnold L. Brown (Chairman), University of Wisconsin Medical School
Joseph Highland, Environmental Defense Fund

William Lijinsky, Frederick Cancer Research Center
Henry Pitot, University of Wisconsin Medical Center
Verne A. Ray, Pfizer Medical Research Laboratory
Verald K. Rowe, Dow Chemical USA
Michael Shimkin, University of California at San Diego
Louise Strong, University of Texas Health Sciences Center
Kenneth Wilcox, Michigan State Health Department

---

* Subsequent to this review, changes may have been made in the bioassay report either as a result of the review or other reasons. Thus, certain comments and criticisms reflected in the review may no longer be appropriate.

☆U.S. GOVERNMENT PRINTING OFFICE: 1979-281-217/3042

DATE DUE

GAYLORD

PRINTED IN U.S.A.

Library
National Institutes of Health
Bethesda, Maryland 20014

LIBRARY
NIH
Amazing Research.
Amazing Help.
http://nihlibrary.nih.gov

10 Center Drive
Bethesda, MD 20892-1150
301-496-1080

NIH LIBRARY
08 4 0120 0319

NIH LIBRARY
3 1496 00123 5350

DHEW Publication No. (NIH) 79-1718